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THE PHYSIOLOGICAL PRODUCTION OF SYMPATHIN IN THE LIVER

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Electrical stimulation of the sympathetic nerve supply of the liver determines the liberation of a cardio-accelerator substance (Cannon and Uridil, 1921; Cannon and Griffith, 1922). This substance has been shown to be physiologically identical with sympathin obtained from other sources (Cannon and Rosenblueth, 1933).

The present study deals with the effects of hepatic sympathin liberated during pseudo-emotional activity of decorticate cats, i.e., under physiological conditions.

METHOD. The following operations were performed aseptically on thirteen cats (in one or two stages): bilateral removal of the upper thoracic sympathetic chains (from T1 to T6), excision of the right and denervation of the left adrenal gland. In four of these animals the hepatic nerves were likewise sectioned.

After full recovery, the animals were made to fast for 24 hours. Under ether anesthesia the cerebral hemispheres were removed. The ether was discontinued and as the activity of sham rage appeared both vagi were cut, completing the denervation of the heart. Continuous records of heart rate were taken by means of a mercury manometer connected with the carotid artery. In five cats, after several typical active periods, the abdomen was opened and the liver nerves were cut.

For the purposes of exposition of the results the following classification of the animals has been adopted: *group 1*, four decorticate animals with the liver nerves intact; *group 2*, five animals in which the liver nerves were severed acutely during the experiment; *group 3*, the four animals with the hepatic nerves sectioned aseptically.

RESULTS. Table 1 summarizes the changes of heart rate recorded. The columns marked "quiet" report the basal heart rate between the fits of

TABLE 1

GROUP	CATS	LIVER NERVES INTACT			LIVER NERVES CUT		
		Heart rate per minute		Per cent acceleration	Heart rate per minute		Per cent acceleration
		Quiet	Active		Quiet	Active	
1	A	192 ± 2	212 ± 8	10.4			
	B	199 ± 1	222 ± 2	11.6			
	C	179 ± 1	194 ± 0	8.3			
	D	199 ± 1	227 ± 3	14.1			
2	E	190 ± 2	192 ± 2	1.0	169 ± 1	170 ± 0	0.6
	F	216 ± 0	224 ± 4	3.7	212 ± 0	217 ± 3	2.4
	G	172 ± 0	187 ± 2	8.7	166 ± 2	173 ± 3	4.2
	H	212 ± 4	221 ± 1	4.3	200 ± 0	204 ± 0	2.0
	I	224 ± 6	242 ± 14	8.0	181 ± 5	188 ± 12	3.9
3	J				176 ± 4	180 ± 10	2.3
	K				219 ± 5	236 ± 8	7.8
	L				241 ± 3	248 ± 0	2.9
	M				172 ± 4	174 ± 6	1.2
Average.....		198	213	7.7	193	199	3.1

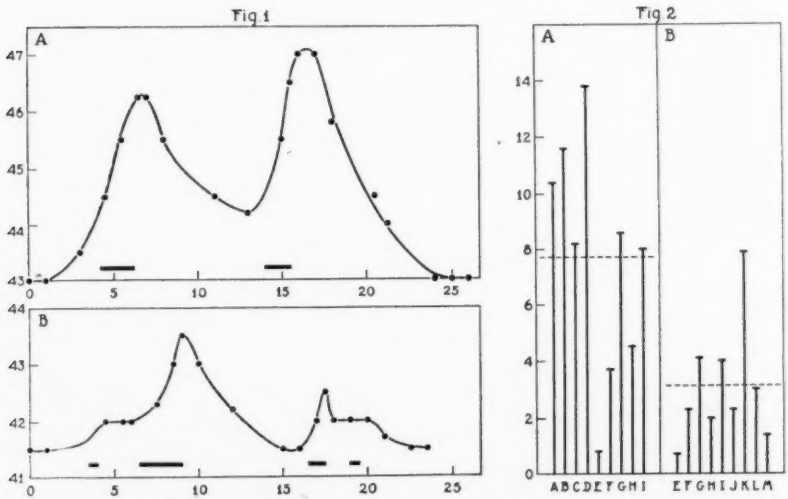

Fig. 1. Typical increments of heart rate attending activity. A, before, and B, after section of the hepatic nerves (cat G, table 1). Ordinates: heart beats per 15 seconds. Abscissae: time in minutes. The signals mark the periods of activity.

Fig. 2. Graphic summary of the accelerations of heart rate A, before, and B, after section of the hepatic nerves. Height of the vertical lines: per cent acceleration per minute. The letters refer to the data in table 1. The two horizontal dotted lines indicate the averages of the corresponding groups.

pseudaffective activity. The columns marked "active" report the maximal heart rate per minute attained after the fits of activity.

Figure 1 illustrates the time course of some typical accelerations induced by activity, *A* before, and *B* after section of the hepatic nerves in cat G of group 2. This time course is similar to that of the responses to electrical stimulation of the hepatic nerves (Cannon and Uridil, 1921).

Figure 2 illustrates graphically the effects of severance of the hepatic nerves on the acceleration attending the fits of activity. The acceleration persisted but was significantly decreased.

Discussion. The basal level of the heart rate in the quiet periods, varying from 166 beats per minute in cat G to 241 in cat L (an extremely vigorous preparation), is considerably above that of the denervated heart in normal cats (120 beats per minute, see Cannon, 1929). In a few instances it was observed that as the active periods of sham rage ceased to occur spontaneously and the cats seemed relatively inert, the heart rates decreased steadily but slowly toward the normal level, although there was no change in body temperature. Probably the intense persistent activity of the sympathetic system in the decorticate animal continuously liberates enough sympathin to maintain the basal rate at these high levels; that the liver is one of the agents involved is shown particularly by cat E, which exhibited accelerations of only one beat per minute both before and after the liver nerves were cut, but showed a striking drop in the basal rate—21.6 beats per minute—when the liver influence was eliminated. A similar drop in basal rate may be observed in other animals in group 2.

In every animal in group 2 acceleration during activity was greater when the liver nerves were intact than when severed (table 1). The accelerations after hepatic denervation (groups 2 and 3) are probably due to sympathin from other sources in the organism. The marked acceleration observed in cat K (table 1 and fig. 2) after severence of the hepatic nerves may have been due to the unusual activity of this animal. It is probable that it might have shown proportionately greater acceleration if the liver nerves had not been cut.

SUMMARY

The effects of section of the liver nerves on the heart rate of decorticate cats with denervated hearts and inactivated adrenals is described (table 1). The time course of typical accelerations induced by activity is similar to that of the responses to electrical stimulation of the hepatic nerves (fig. 1). The average acceleration attending pseudo-emotional activity when the liver nerves were intact was 2.5 times as great as it was after the nerves were severed (fig. 2). The basal heart rate decreased significantly on section of the hepatic nerves (table 1).

From these facts the conclusion is drawn that sympathin is liberated from the liver under physiological conditions such as those of the decorticate animal.

We wish to express our appreciation to Dr. Arturo Rosenblueth who conceived and directed these experiments.

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A STUDY OF THE INTRAGASTRIC FACTORS IN THE REGULATION OF GASTRIC ACIDITY

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In a previous communication Wilhelmj, Neigus and Hill (1934) presented evidence which seemed to indicate that the stomach itself possesses very little ability to reduce the acidity of acid gastric contents. The evidence for this conclusion was somewhat indirect and Dr. A. C. Ivy suggested that it should be verified by similar studies on isolated whole stomach pouches. The present communication presents the results of these studies.

Six whole stomach pouches were used which differed slightly in type. In one the entire stomach was included in the pouch and the esophagus anastomosed to the duodenum by the two stage technique of Mann. This animal failed to remain in a satisfactory condition during the month it was under observation and only one complete experiment was performed on it. In two animals a small part of the cardiac end of the stomach (about 1 inch in length and including the entire width of the stomach at this level) was left attached to the esophagus and joined to the duodenum by an end to side anastomosis. These two animals remained in excellent condition and were kept for several months during which time many experiments were performed. In both of the above types of pouch it was necessary to cut most of the fibers of the vagus nerve going to the pouch. In order to exclude this partial denervation of the pouch as a factor in our results, we prepared three additional pouches in which the vagus nerve supply of the pouch was left intact. These pouches were prepared by a two stage operation as follows: At the first operation the duodenum was severed from the pylorus and both were closed, at the same time an anterior gastrojejunostomy was performed. After the lapse of about 6 weeks, when the animals were in excellent condition, the jejunum was removed from the stomach and the opening in the jejunum closed; the opening into the stomach was then brought into the wound and firmly sutured. The whole stomach was thus detached from the intestine but attached to the esophagus and communicated to the outside by a gastrostomy; the vagus supply from above was intact. The two stage technique was employed in

order to allow the pyloric end to heal completely before using. No experiments were performed on the day following the second operation but the animal was given 900 cc. of physiologic saline solution subcutaneously. Experiments were performed on the 2nd, 3rd, and 4th post-operative days and after each experiment 900 cc. of saline solution were given subcutaneously. Two of these animals remained in excellent condition during the experimental period and showed no untoward symptoms whatever and can be considered as quite normal except for the necessary period of fasting. As regards the ability to reduce the acidity of hydrochloric acid solution introduced into them, there was no essential difference between the partially denervated and the non-denervated pouches. Only one major difference was noted which will be referred to later.

METHODS. The general method of procedure was to introduce approximately 150 cc. of a standardized hydrochloric acid solution (approximately one-tenth normal) containing phenol red into the pouch and allow it to remain for one-half hour; at the end of the half-hour period the pouch was emptied and a second 150 cc. quantity of the acid-phenol red solution introduced. In some experiments the pouch was stimulated by an intramuscular injection of histamine and three one-half hour samples obtained after histamine injection, while in other experiments no histamine was used. Before starting an experiment the pouch was always thoroughly lavaged with several changes of the acid-phenol red solution to be used, the total amount used in lavaging was usually from 300 to 450 cc. This was done in order to remove mucus and accumulated secretions and to allow the experiment to start from an approximate zero base line.

During an experiment the opening into the pouch was closed with a proper fitting rubber stopper, through which a soft rubber catheter was passed. The catheter was not allowed to remain in the pouch during an experiment, since it was desired to prevent mechanical stimulation of the gastric mucosa. Withdrawing and introducing of the catheter without loss of solution was accomplished by means of a small piece of glass tubing which passed through the stopper and was tipped at its outer end with a short piece of rubber tubing. After introducing the acid solution into the pouch the catheter was withdrawn from the pouch into the rubber tubing and clamped with a hemostat.

The following analyses were performed on the acid solution removed from the pouch: 1. The per cent of phenol-red after removing the proteins with 10 per cent sodium tungstate and two-thirds normal sulfuric acid. 2. Total and neutral chloride after careful ashing of the dried sample.

The per cent of phenol-red present in the sample showed how much fluid was added to the sample while in the stomach and allowed the original acid solution introduced into the pouch to be corrected for dilution. With this information it was possible to determine the composition of the

gastric secretions added to the acid solution while in the pouch. The methods and calculations have been described in detail in previous publications (1933, 1934).

RESULTS. *A. Experiments without histamine stimulation.* Only those experiments in which there was no evidence of secretion of acid by the pouch have been considered under this heading. A very interesting difference was found between the partially denervated (vagus) pouches and the non-denervated pouches. The former usually showed no evidence of secretion of acid when filled with the hydrochloric acid solutions while the latter usually secreted fairly large amounts of acid. In this respect the non-denervated stomach which has been severed from the duodenum is quite different from the intact stomach, in which we have performed many experiments without histamine stimulation and which usually shows no evidence of secretion of acid. This difference between the intact stomach with and without connection to the duodenum, suggests that the intestine may normally exert an inhibiting influence on the acid secreting mechanism of the stomach. However, Ivy, Lim and McCarthy (1925) in their studies on the intestinal phase of gastric secretion found that hydrochloric acid in the intestine might either stimulate or inhibit gastric secretion, the stimulating effect being more common. In a personal communication Doctor Ivy suggests that the hypersecretion observed by us may depend upon the recent operation since he frequently observes a post operative hypersecretion after upper abdominal operations.

The results of 15 one-half-hour experiments are shown in table 1. The results are given both as per cent and as the *total amount* of the various constituents present in the entire sample. The latter figures are particularly important in the present problem since the total amounts of the various constituents may be compared with those previously found in fundic pouches and in pouches of the whole pyloric portion of the stomach. Comparison on a percentage basis would be without value because of the different volumes of solution used (20–30 cc. in pyloric and fundic pouches and 150 cc. in whole stomach pouches).

The following points deserve special emphasis:

1. *The reduction in acidity* which occurred during the half-hour period was in general very small. This is evident without mathematical analysis of the data. The acid solutions introduced into the pouch contained approximately 350 mgm. of acid chloride per 100 cc. (col. 3) while the average acid chloride concentration of the samples removed from the pouch was 322 mgm. per 100 cc. This is the total reduction in acid chloride concentration and is the combined result of neutralization and dilution.

2. *The total amount of neutral chloride* in the samples removed from the pouches averaged 32 mgm. (col. 13) with maximal and minimal values of 42 and 17 mgm. respectively. If the entire quantity of neutral chloride

TABLE 1

Results of experiments on whole stomach pouches in which acid solutions were introduced into the pouch and allowed to remain for one-half hour

The pouches were not stimulated with histamine and there was no evidence of secretion of acid. In most experiments the amount of acid solution introduced was 150 cc. In the last two experiments on dog I the amounts were 150, 100, and 50 cc. respectively for the 1st, 2nd, and 3rd half-hour periods. In these experiments it should be noted that due to the decreasing volumes there appears to be a progressive elevation of neutral chloride when expressed as mgm. per 100 cc. (col. 7); whereas, the actual amount of neutral chloride in the sample (col. 13) is nearly constant.

Both pouches are the partially (vagus) denervated type as described in the text.

DOG	LENGTH OF EXPERIMENT, HOURS	CHLORIDE OF ACID SOLUTION, MGM. PER 100 CC.	PHENOLSULPHONEPHTHALEIN, PER CENT	CHLORIDE OF ORIGINAL ACID SOLUTION CORRECTED FOR DILUTION	MILLIGRAMS PER 100 CC.					VOLUME OF SAMPLE, CC.	AMOUNT ACTUALLY PRESENT IN SAMPLE				CHLORIDE CONCENTRATION OF SECRETION, MGM. PER 100 CC.	CHLORIDE OF NEUTRALIZED ACID / CC. OF SECRETION	NORMALITY	NEUTRAL CHLORIDE / CC. OF SECRETION
					Total chloride	Neutral chloride	Total minus neutral chloride	Extra chloride	Acid chloride ± corrected value		Extra chloride	Neutral chloride	Acid chloride ± corrected value	Amount of secretion, cc.				
II	½	349	98	342	351	15	336	9	−6	180	16	27	−11	4	450	3.0	0.08	7.5 (5.0)
	½		98	342	348	15	333	6	−9	181	11	27	−16	4	300	4.5*	0.126*	7.5 (4.3)
	½		98	342	351	9	342	9	0	190	17	17	0	4	450			4.5 (4.5)
I	½	351	93	326	351	30	321	25	−5	128	32	38	−6	9	357	0.7	0.02	4.3 (3.8)
	½		96	337	354	26	328	17	−9	154	26	40	−14	6	425	2.3	0.06	6.5 (4.7)
	½		96	337	348	24	324	11	−13	155	17	37	−20	6	275	3.2	0.09	6.0 (3.8)
I	½	351	93	326	347	21	326	21	0	154	32	32	0	11	300	0.0		3.0 (3.0)
	½		96	337	351	17	334	14	−3	157	22	27	−5	6	350	0.8	0.02	4.3 (3.8)
	½		96	337	349	19	330	12	−7	153	18	29	−11	6	300	1.8	0.05	4.8 (3.7)
I	½	351	91	320	343	23	320	23	0	156	36	36	0	14	256	0.0		2.6 (2.6)
	½		93	327	348	28	320	21	−7	105	22	29	−7	7	300	1.0	0.03	4.0 (3.4)
	½		89	313	348	46	302	35	−11	53	19	25	−6	6	319	1.0	0.03	4.2 (3.6)
I	½	351	94	330	345	21	324	15	−6	159	24	33	−10	10	250	1.0	0.03	3.5 (3.0)
	½		89	312	348	39	309	36	−3	108	39	42	−3	12	328	0.3	0.01	3.6 (3.4)
	½		88	309	352	66	286	43	−23	55	24	36	−13	7	358	1.1	0.03	5.5 (4.1)
Average							322				24	32	−8	8	335	1.5	0.04	4.8 (3.8)

* Not included in average.

was the result of neutralization of hydrochloric acid it would represent only an average of 9 cc. of tenth normal hydrochloric acid neutralized by the whole stomach during one-half hour. However, not all of the neutral chloride can be ascribed to neutralization of hydrochloric acid since

a certain amount entered with the non-acid secretions of the stomach-(pyloric secretion and fundic mucus). Of the total amount an average of only 8 mgm. actually represents neutralized hydrochloric acid (col. 14) while 24 mgm. entered with the non-acid secretions of the pouch (col. 12). Thus in the whole stomach under the conditions of these experiments, only about one-fourth of the neutral chloride represents neutralized acid.

In previously reported studies (1934) on pouches of the entire pyloric region it was found that the total neutral chloride averaged 19 mgm. with maximal and minimal values of 38 and 8 mgm. respectively. This comparison suggests that in the whole stomach approximately one-half of the neutral chloride is contributed by the pyloric region and one-half by the fundic region.

3. *The actual amount of secretion by the stomach* during the one-half hour period averaged 8 cc. (col. 15) with maximal and minimal amounts of 14 and 4 cc. respectively. Pyloric pouches under similar conditions were found to secrete an average of 4 cc. per half hour with maximal and minimal amounts of 2 cc. and 7 cc. This suggests that approximately one-half of the non-acid secretion of the whole stomach is contributed by the pyloric region and one-half by the fundic region.

4. *The neutral chloride concentration of the non-acid secretion of the whole stomach* averaged 335 mgm. per 100 cc. (col. 16) and ranged from 250 to 450 mgm. per 100 cc. Pyloric secretion was previously shown (1934) to average approximately 376 mgm. per 100 cc., under similiar experimental conditions.

5. *The alkalinity of the non-acid secretion of the whole stomach* was found to average 0.04 normal (col. 18.) The same average alkalinity was previously reported for the secretion of pyloric pouches (1934). Bolton and Goodhart (1933) have reported the same average value for the alkalinity of gastric mucus

6. *The ratio of neutral chloride to the cubic centimeters of secretion* shows the amount of neutral chloride related to each cubic centimeter of secretion, it includes the neutral chloride content of each cubic centimeter of secretion plus the neutral chloride due to neutralization of hydrochloric acid by each cubic centimeter of secretion. This ratio averages 4.8 (col. 19). In order to make this ratio more comparable to that obtained when the stomach is secreting hydrochloric acid it has been corrected by assuming that the acid neutralized contained 578 mgm. of acid chloride per 100 cc. and adding the fluid of the neutralized acid to the cubic centimeter of secretion. The corrected ratios are shown in parenthesis and average 3.8. The significance of this ratio will be referred to later.

B. *Experiments with histamine stimulation.* After thoroughly lavaging the pouch with the acid-phenol red solution, 150 cc. were placed in the pouch and one or two milligrams of histamine given intramuscularly. At

the end of one-half hour the pouch was emptied and 150 cc. of fresh solution introduced. Three one-half-hour samples were obtained after the injection of histamine.

A typical set of experiments is shown in table 2. Pouches I and II are the partially denervated type, while pouches III and IV are the non-denervated type in which the stomach was still attached to the esophagus but disconnected from the duodenum. The results in table 2 are given as the *total amount* of the various constituents present in the sample and not as *per cent.*

1. *Chloride of the acid secreted* is shown in column 7. Secretion of acid is seen to begin in the first half hour, reach its peak in the second half hour and drop rapidly in the third half hour. This curve is in every way similar to that found in the normal intact stomach (1933–1934).

2. *Neutral chloride* (col. 8). In practically all experiments the neutral chloride was highest in the first half-hour sample and fell progressively in the remaining samples; this was probably due to the fact that the thick, tenacious mucus which covers the resting mucosa was not completely washed out before starting the experiment but was gradually washed out as secretion proceeded. From these results it is clear that the neutral chloride does not increase as the acid chloride falls, hence the inverse relationship between acid chloride and neutral chloride, often described as a typical finding in the intact, normal stomach (Bolton and Goodhart, 1922; Roseman, 1907) is obviously not a function of the gastric mucosa. The total neutral chloride in the 33 experiments shown in table 2 averaged 24 mgm. while the maximal and minimal values were 48 and 10 mgm. respectively; these values are approximately the same as those given in table 1 where histamine stimulation was not used. Since the chloride of any neutralized hydrochloric acid would be present as neutral chloride it is clear that under the conditions of these experiments the amount of acid neutralized by the whole stomach is relatively small and is approximately the same with and without histamine stimulation.

3. *The total fluid entering the stomach* during the half-hour period is shown in column 9. Wilhelmj, Neigus and Hill (1934) have shown that because of the approximate constancy of the chloride concentration of the secretion of the fundic portion of the stomach, it is possible to separate the total fluid entering the stomach into two fractions: *a*, the fluid of hydrochloric acid which is secreted and not neutralized (col. 10), and *b*, the "extra fluid" (col. 11) which in these isolated whole stomach pouches is composed of pyloric secretion, fundic mucus and the fluid of acid which is secreted and subsequently neutralized. In making this calculation we have divided the milligrams of acid chloride secreted by the stomach, but not neutralized, by 5.78 which gives the cubic centimeters of "acid fluid;" the difference between this value and the total fluid entering the stomach

TABLE 2

Results of experiments on whole stomach pouches in which acid solutions (approximately 150 cc., were introduced into the pouch and allowed to remain for one-half hour)

The pouches were stimulated by an intramuscular injection of histamine. Pouches I and II are the partially denervated type (vagus) while III and IV are the non-denervated type as described in the text.

DOG	TIME AFTER HISTAMINE, HOURS	CHLORIDE OF ACID SOLUTION, MGM. PER 100 CC.	PHENOLSULPHONEPHTHALEIN, PER CENT	VOLUME OF SAMPLE, CC.	EXTRA CHLORIDE, MGM.	ACID CHLORIDE = CORRECTED VALUE, MGM.	NEUTRAL CHLORIDE, MGM.	TOTAL FLUID ESTERIFIED STOMACH, CC.	FLUID OF SECRETED ACID, CC.	EXTRA FLUID, CC.	NEUTRAL CHLORIDE CC. OF EXTRA FLUID	CHLORIDE CONCENTRATION OF SECRETION, MGM. PER 100 CC.	REMARKS
I	½	351	88	170	113	+65	48	20	11	9	5.3	550	2 mgm. histamine
	1		82	187	189	+150	39	34	26	8	4.9	560	
	1½		90	164	82	+54	28	16	9	7	4.0	500	
I	½	351	90	162	73	+28	45	16	5	11	4.1	450	1 mgm. histamine
	1		91	164	77	+49	28	15	9	6	4.7	524	
	1½		96	155	19	0	19	6	0	6	3.2	300	
I	½	351	89	166	86	+51	35	18	9	9	3.9	473	1 mgm. histamine
	1		86	173	121	+102	19	24	18	6	3.2	500	
	1½		97	152	18	+3	15	5	0.5	4.5	3.3	400	
I	½	351	89	152	70	+41	29	17	7	10	2.9	418	2 mgm. histamine
	1		85	179	143	+118	25	27	20	7	3.6	534	
	1½		93	158	51	+38	13	11	7	4	3.3	458	
I	½	351	90	164	64	+30	34	16	5	11	3.1	390	2 mgm. histamine
	1		87	169	108	+85	24	22	15	7	3.4	493	
	1½		98	156	6	−8	14	3	0	3	4.7	200	
I	½	351	89	215*	97	+65	32	24	11	13	2.5	409	1 mgm. histamine
	1		91	162	65	+52	13	15	9	6	2.2	445	
	1½		95	152	23	+11	12	8	2	6	2.0	300	
Average							26			7	3.6		
II	1½	349	90	152	82	+72	10	15	12	3	3.3	550	2 mgm. histamine
II	½	349	90	151	88	+69	18	15	12	3	6.0	580	2 mgm. histamine
	1		81	176	200	+178	23	34	30	4	5.8	600	
	1½		91	156	80	+67	13	14	12	2	6.5	567	
II	½	349	89	163	110	+78	31	18	13	5	6.2	610	2 mgm. histamine
	1		79	181	218	+187	31	38	32	6	5.2	572	
	1½		83	170	163	+143	20	29	25	4	5.0	565	
II	½	349	91	146	69	+50	19	13	9	4	4.8	523	2 mgm. histamine
	1		82	164	172	+150	23	30	26	4	5.7	585	
	1½		93	145	49	+38	12	10	7	3	4.0	485	
Average							20			4	5.3		
III	½	348	86	172	141	+102	40	24	18	6	6.8	586	1 mgm. histamine
	1		91	160	79	+58	21	14	10	4	5.2	544	
	1½		93	159	48	+29	19	11	5	6	3.2	429	
IV	1	348	91	157	74	+47	27	14	8	6	4.5	522	0 histamine
	1½		91	160	70	+43	27	14	7	7	3.9	489	
Average							28			6	4.7		
Average of 33 experiments							24			6	4.3		

*Two hundred cubic centimeters introduced.

gives the cubic centimeters of "extra fluid." The value of 578 mgm. for the chloride concentration of fundic secretion was that obtained by Wilhelmj, Neigus and Hill (1933). The recent studies by Hollander show that pure parietal secretion probably contains no neutral chloride and that the chloride concentration of the hydrochloric acid as secreted is approximately 604 mgm. per 100 cc., hence it would possibly be more exact to divide the milligrams of secreted acid chloride by 6.0 rather than by 5.78; however, the difference introduced by this correction would be very small and would not alter our general conclusions. In the 33 experiments shown in table 2 the total amount of "extra fluid" secreted by the stomach

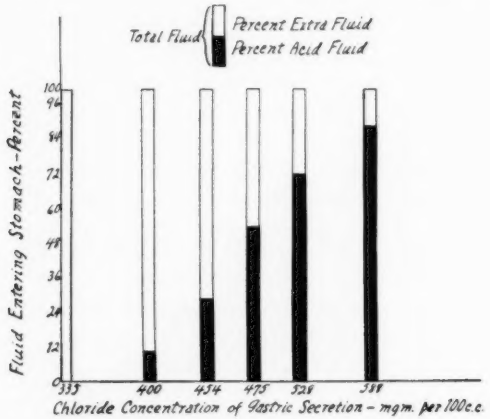

Fig. 1. An analysis of 49 experiments performed on four whole stomach pouches showing that the chloride concentration of gastric secretion is dependent upon the relative amounts of acid and extra fluid present in the mixed secretion. Values obtained by averaging groups of samples containing progressively increasing percentages of acid fluid.

averaged 6 cc. per half hour and varied between 2 and 13 cc. in individual experiments. The extra fluid in these experiments with histamine stimulation should theoretically be identical with the non-acid secretion of the non-stimulated stomach shown in table 1. The quantity of extra fluid is approximately the same as the quantity of non-acid secretion of the non-stimulated stomach. The neutral chloride—extra fluid ratio (col. 12) shows the amount of neutral chloride related to each cubic centimeter of extra fluid and includes the neutral chloride content of the extra fluid as well as the neutral chloride arising from the neutralization of hydrochloric acid by each cubic centimeter of extra fluid. The rather wide fluctuations of this ratio are not surprising in view of the fact that it is influenced by the

summated experimental errors; however, the average values for each pouch are of the same order of magnitude as the ratio of the neutral chloride to the cubic centimeter of secretion in the non-stimulated pouches. The similarity of the amounts and of the ratios lends conviction to the assumption that the extra fluid in the stimulated stomach is the same as the non-acid fluid secreted by the non-stimulated stomach.

4. *The chloride concentration of gastric secretion* (col. 13). Since gastric secretion is composed of two distinct fractions of different chloride concentration (the non-acid secretion averaging approximately 335 mgm. of chloride per 100 cc. and the acid secretion containing between 578 and 604 mgm. of chloride per 100 cc.) it is obvious that the chloride concentration of the mixed secretion will depend upon the *relative* amounts of these two secretions. This is shown in figure 1 where it is seen that the average chloride concentration of gastric secretion may vary from 335 mgm. to 588 mgm. per 100 cc. depending upon the per cent of acid fluid present. When no acid is present the lower value will be obtained but it will rise progressively as the percentage of acid fluid increases. Figure 1, made from experiments on the isolated, whole stomach should be compared with a similar figure made from experiments on the intact stomach in which extra fluid of duodenal origin also entered the stomach. (Wilhelmj, Henrich, Neigus and Hill, 1934.) The general similarity of the two figures is due to the fact that, under the conditions of these experiments, the chloride concentration of the duodenal secretions is approximately the same as that of the non-acid secretion of the whole stomach.

Discussion. The results reported in this paper show quite clearly that under these experimental conditions, the whole stomach has relatively little ability to reduce the acidity of acid solutions.

It is interesting to compare these experiments on whole stomach pouches with experiments done on the intact stomach in which there was no bile present (zero Pettenkoffer reaction) (Wilhelmj, Neigus and Hill, 1934). The experiments on the intact stomach were performed in exactly the same manner and histamine stimulation was used. In 17 such experiments the *total amount* of extra fluid per half hour averaged 17 cc. with variations ranging between 8 and 30 cc. The *total amount* of neutral chloride averaged 73 mgm. with variations ranging from 48 to 112 mgm. The greater amount of extra fluid and neutral chloride found in the intact stomach connected to the duodenum suggests either that the two sets of experiments are not comparable, or that small amounts of duodenal secretions containing practically no bile may at times regurgitate into the stomach. The latter possibility was stressed many years ago by Spencer, Meyer, Rehfuss and Hawk (1915) and appears quite likely in view of the recent observation reported by Ivy (1933).

SUMMARY

Under the conditions of these experiments we find that

1. The whole stomach possesses very little ability to lower the acidity of acid solutions introduced into it.

2. The secretion of the whole stomach is composed of two fractions: *a*, the acid secretion which has a chloride concentration ranging between 578 and 604 mgm. per 100 cc., and *b*, the non-acid secretion having a chloride concentration of approximately 335 mgm. per 100 cc.

3. The non-acid secretion of the stomach is rather limited in quantity, the amounts varying from 2 to 14 cc. for half-hour periods. The amount of the non-acid secretion is apparently independent of the amount of acid secretion.

4. The average alkalinity of the non-acid secretion was found to be 0.04 normal.

5. The total amount of neutral chloride found in the isolated stomach is rather small in amount and does not rise as the acid secretion falls.

6. These studies indicate that the most important intragastric factor in the regulation of gastric acidity is the intensity of the stimulus for acid secretion.

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SODIUM AND CHLORIDE IN FROG MUSCLE

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A general theory of the behavior of electrolytes in muscle has been proposed by Mond and Netter (1930, 1932) according to which the membrane is permeable to potassium and impermeable in general to anions and to cations of larger effective diameter than potassium. Some evidence in favor of this theory was presented by us in a recent study of the diffusion of potassium in and out of frog muscles when soaked in various solutions (Fenn and Cobb, 1934). This evidence might be described as good if not compelling. However, similar evidence from studies of mammalian muscles in vivo is not available on account of the difficulty in distinguishing between electrolytes in tissue spaces and in the fibres themselves. In continuing this study, therefore, we wish to keep our minds open and to look for further evidence of cation permeability and anion impermeability without regarding the matter as entirely settled.

The work reported in this paper consists of studies of the sodium chloride content of frog muscle analyzed both before and after soaking in various solutions. The results show that after soaking, both sodium and chloride exist in frog muscle in concentrations very nearly directly proportional to the concentration in the outside solution. In muscles in intact frogs, however, there is always an excess of sodium of variable amount, that is, more sodium in relation to the chloride than can be accounted for by the sodium to chloride ratio in plasma. This excess sodium largely disappears on soaking in Ringer's solution. This represents, therefore, further confirmation of the conclusions of Mond and Netter regarding the state of sodium in muscle.[1] We have, however, no evidence regarding the function of this excess sodium or the anions with which it is combined.

METHODS. For chloride determinations in plasma we used the micro open-Carius method as described by Peters and Van Slyke (1932). Where sufficient amounts of muscle were available (1 gm.) we used the method of Van Slyke (1923). For muscles soaked in different solutions we used the

[1] It should be mentioned that these authors calculated the excess sodium in muscle on the basis of the sodium to chloride ratio in blood instead of plasma so that their figures appear to be in error to this extent.

sartorius muscle, which is thin enough to permit adequate diffusion. The small size of this muscle necessitates a more sensitive method and we used the method designed by Doctor Westfall and kindly communicated to us by Professor A. N. Richards.[2] This method consists of making a water extract of the muscle after cutting it into fine pieces with scissors. The suspension of about 100 mgm. of muscle in 4 cc. of water is shaken in a test tube for an hour, filtered out, and solid Ag_2CrO_4 added to the filtrate. The chloride present exchanges with chromate and a corresponding amount of chromate is liberated in solution. The suspension is filtered through a no. 5 Whatmann filter and the chromate determined colorimetrically by the addition of s-diphenylcarbazide.

In studying these chloride methods a series of ten frogs was used. The two sartorius muscles were analyzed for chloride separately by the Westfall method while two samples of the leg muscles, each weighing about 1 gram, were analyzed by the Van Slyke method. The average values obtained were 1.08 m.-eq. per cent by the Westfall and 1.09 m.-eq. per cent by the Van Slyke method. The average difference between two results by the same method was 9 per cent while the average difference between two results by different methods was 12 per cent. The methods themselves were more accurate than this, these large differences being due to inequalities between different muscle samples as to the amount of blood included, etc. There seems to be, however, no systematic error in either method except that the Westfall method may leave as much as perhaps 1 per cent of the chloride unextracted from the muscle. Analyses of extracted muscle residues for chloride showed only traces remaining.

In general we used sartorius or thigh muscles for our analyses. Gastrocnemius muscles frequently gave lower chloride values, probably because of the greater amount of tendon. In seven winter frogs with an average chloride content of 1.02 m.-eq. per cent in thigh muscles, the gastrocnemius contained only 0.85 m.-eq. per cent as an average.

Sodium was determined by Salit's modification (1932) of the method of McCance and Shipp (1931). The tissue was first dry ashed in a platinum crucible in an oven at not over 650°C. for 15 hours. Control determinations showed that no sodium was lost from known solutions during this treatment. In some experiments the muscle was first immersed in sulfuric acid and evaporated to dryness before ashing. Control muscles similarly ashed without sulfuric acid treatment showed no lower sodium values, indicating that transformation to sulfates was not necessary to prevent loss of sodium in our oven.

Blood samples were taken from frogs after their brains had been crushed with a strong hemostat. A syringe needle was tied into the aorta so that the heart pumped its blood directly into the syringe. Clotting was pre-

[2] This method is shortly to be published in the Jour. of Biol. Chem.

vented by heparin and the blood was oxygenated before being centrifuged; 0.217 cc. of plasma in a blood-counting pipette sufficed for either sodium or chloride analyses.

RESULTS. Sodium and chloride contents of normal frog muscles at different seasons of the year are shown in table 1. Some of these frogs were bled before the muscles were dissected as indicated, and this caused a 5 per cent decrease in the values obtained for chloride (8 comparisons). In general it may be said that sodium values fluctuated insignificantly at

TABLE 1

Sodium and chloride contents of frog muscles

(Milli-equivalents per 100 grams)

MONTH	NUMBER OF ANALYSES	Na	PROBABLE ERROR
August–September	11	2.37	0.32
October	10	2.46	0.14
November	8	2.64	0.31
January	16	2.35	0.21*
February	10	2.79	0.22*
March	11	2.54	0.49*
April	3	2.45	0.26
		Cl	
January	21	0.81	0.19*
February	6	0.87	0.05*
March	15	1.12	0.13*
April–May	8	0.96	0.09
May–June	9	1.05	0.21*
June	5	1.37	0.38
August–September	7	1.65	0.44
July	8	0.73	0.14*
Grand average Sodium		2.54	
Grand average Chloride		1.09	

* Analyses in 1934, others in 1933; frogs bled before dissection, Van Slyke method used for chloride analyses, otherwise the Westfall method. July chloride analyses not included in the grand average.

different seasons of the year while the chloride values varied from 0.73 m.-eq. per 100 grams to 1.65, with, however, a rather large probable error. The condition of the frog is probably more important in this respect than the season of the year. Average values for sodium and chloride, respectively, were 2.54 and 1.09 m.-eq. per cent, that is, more than twice as much sodium as chloride. Since the average ratio of sodium to chloride in the plasma was found to be 1.36, there is obviously relatively more sodium in muscle than in plasma, the difference being "excess sodium."

The relative amounts of sodium and chloride in plasma of frogs are shown in figure 1. Plasmas from normal frogs are represented by dots and show an average ratio of sodium to chloride of 1.36. In order to study variations in this ratio some frogs were left for 24 to 48 hours in Ringer's solution or hypertonic Ringer's solution.[3] Some others were left in the cold room in distilled water for varying periods. As indicated in figure 1 by triangles, these cold room frogs showed in general abnormally low chloride values, while the frogs soaked in hypotonic (crosses), isotonic (circles) or hypertonic (crosses in circles) solutions showed abnormally high chloride

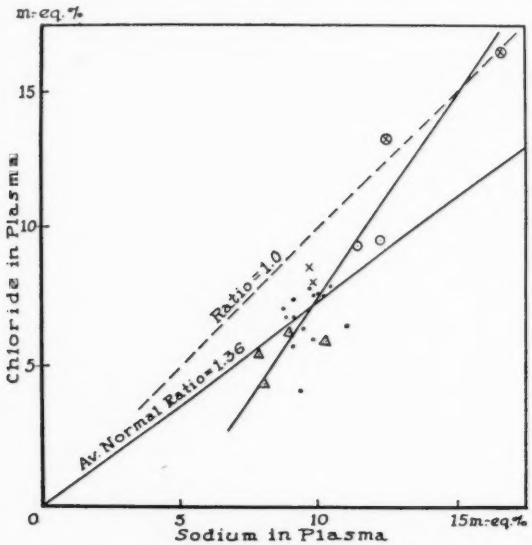

Fig. 1. Comparison of chloride and sodium contents of plasma in winter frogs in m.-eq. per 100 cc. Normal frogs indicated by ●, cold room frogs (1–10 days) by △, frogs in 1 part Ringer plus 2 parts water by ×, frogs in Ringer by ⊙, and frogs in 1½ or 2 times hypertonic Ringer by ⊗.

values in relation to sodium. It may be concluded from this diagram that the sodium content of plasma is relatively more stable than the chloride content and that soaking in hypertonic solutions tends to increase the chloride more than the sodium so that the sodium to chloride ratio approximates that of the solution where it is nearly equal to 1.0.[4]

[3] Our Ringer's solution contains 0.65 per cent NaCl, 0.01 per cent KCl, 0.02 per cent $CaCl_2$ and $\frac{m}{150}$ phosphate buffers at pH 7.25.

[4] We do not wish to stress unduly the low chloride values (compared to sodium) found in plasma of cold room frogs, since our series of four frogs is rather limited.

In the same frogs used for the plasma analyses of figure 1 determinations were also made of the sodium and chloride contents of muscles and the results are plotted in figure 2. As before, normal muscles were represented by dots, muscles from frogs in hypertonic solutions by circles. If the sodium to chloride ratio in muscle were the same as in plasma the experimental points should lie along the diagonal marked 1.36. Actually all the points lie below this diagonal but along a line roughly parallel to it. It appears that if 1 m.-eq. of sodium per 100 grams were subtracted from all the sodium values, both for normal and hypertonic frogs, the ratio of sodium to chloride would have been approximately equal to that in plasma. This indicates that the excess sodium in frogs immersed in hypertonic

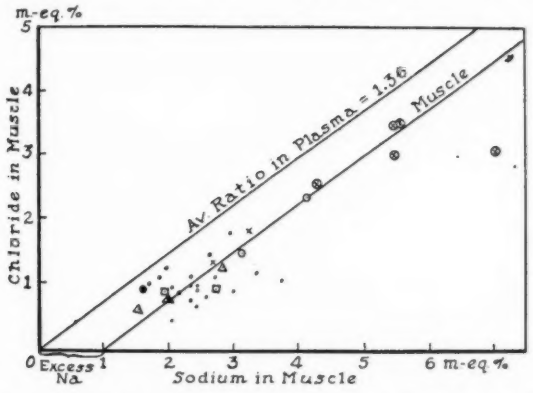

Fig. 2. Comparison of chloride and sodium content of muscles of frogs used for figure 1, and a few others. Values in m.-eq. per 100 grams wet weight. Muscles from normal frogs are represented by ●, frogs which revived after being frozen by △, frogs kept at 4°C. by ⊡, frogs in ⅓ Ringer plus ⅔ water by ×, frogs in Ringer by ⊙, and frogs in 1½ or 2 times concentrated Ringer by ⊗. One large dot represents the average value given by Mond and Netter for normal frog muscle.

Ringer remains fairly constant and in all cases is about 1 m.-eq. per 100 grams. It will be shown later that this is not true of muscles soaked in Ringer's solution after dissection.

On the assumption that the sodium and chloride are located in the interspaces between the fibres and further, that they are present in these inter-

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Muscle chloride was not abnormally low in three of these frogs, but the fourth had only 0.28 m.-eq. per cent and the plasma only 4.4 m.-eq. per cent. Sartorius muscles from this frog when soaked 5 hours in Ringer's solution lost 12 per cent in weight but gained nine-fold in chloride content. Two frogs which came into the laboratory frozen stiff in transit, but which subsequently revived, were studied from this point of view but only one of them gave low chloride values in plasma.

spaces in concentrations equal to their concentrations in plasma, the volume of these interspaces may be calculated from the ratio of the concentration in muscle to the concentration in plasma. This ratio may be shown diagrammatically by plotting the sodium and chloride content in muscle against the sodium and chloride content in plasma as in figure 3. Sodium values are represented by circles and chloride values by crosses. Straight diagonal lines represent the average normal ratio of muscle to plasma concentrations. Normally 14.5 per cent of the muscle contains chloride and 24.2 per cent contains sodium in concentrations equal to the concentrations in plasma. The higher value for sodium is a further indication of an excess sodium in muscle. Figure 3 includes also values obtained from muscles soaked in hypertonic Ringer's solutions. To avoid confusion these points

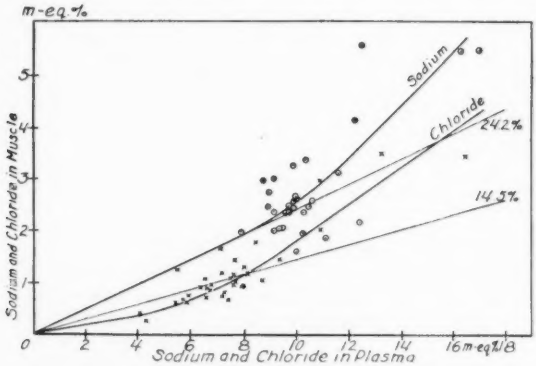

Fig. 3. Comparison between the muscle and plasma contents of sodium and chloride. Sodium analyses represented by ⊙ and chloride analyses by ×. Muscles were analyzed immediately after dissection. High contents were obtained by soaking the frogs in Ringer and hypertonic Ringer.

are not especially distinguished from normal points. It is evident, however, that in the hypertonic frogs the muscle content in both sodium and chloride increases faster than the sodium and chloride content in plasma. Points from hypertonic frogs do not lie along the normal diagonals 24.2 and 14.5 per cent but along curves which are distinctly concave upwards. This may be interpreted as meaning that the tissue spaces are increasing in volume with respect to the muscle fibres themselves. Probably some fibres are breaking down, liberating potassium and taking in NaCl.

In order to produce other experimental variations in the sodium and chloride content of muscles, sartorius muscles were immersed in solutions of varying chloride and varying sodium contents for 5 hours at 22°C. Control muscles were analyzed in most cases before soaking. A series of

observations of this sort for chloride is plotted in figure 4. The chloride content of the solution was altered by replacing varying amounts of sodium chloride by sodium nitrate or by adding NaCl to hypertonicity. It was found that the chloride content of the muscles after 5 hours was regularly about 31 per cent of the concentration of chloride in the solution; that is, the experimental points fall along a diagonal as indicated in the figure. In these experiments one muscle was always soaked in Ringer's solution while the control muscle was soaked in some weaker or stronger solution. To avoid confusion no indication is given in figure 4 which points represent paired muscles of the same frog except in two cases where the paired points are connected by dotted lines. These lines have approximately the same slope as the 31 per cent diagonal but are widely displaced above and below it due to individual differences between frogs. Had we plotted merely

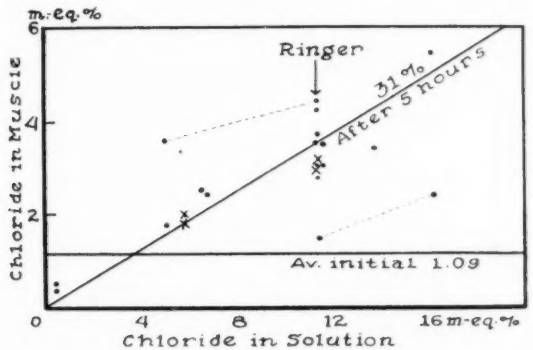

Fig. 4. Chloride content of frog sartorius muscles after soaking for 5 hours at 22°C. in solutions of different chloride content. Results of two later experiments are shown by crosses. Calculations based on weight after soaking.

gain or loss of chloride in per cent of the content in Ringer's solution these points would have coincided much better with the general curve. The average chloride content of muscles soaked in Ringer's solution (3.6 m.-eq. per cent) compared with the average initial value of 1.09 m.-eq. per cent shows that muscles immersed in Ringer's solution regularly gain large amounts of chloride, until they contain over three times the normal amount. We cannot as yet definitely account quantitatively for all this influx of chloride in terms of other cations gained or other anions lost.

Similar experiments with sodium are plotted in figure 5. In this case the sodium content of the solution was varied by replacing sodium chloride by 4.5 per cent dextrose. Again the experimental points fall along a diagonal, the concentration in the muscle being regularly about 33 per cent of the concentration in the solution. This is very nearly the

same ratio as the 31 per cent found for chloride, and it may be concluded that after soaking in solutions the muscles contain sodium and chloride in one-third the volume of the muscle in amounts equal to the concentrations in the solution. It may be seen that in general muscles also gain slightly in sodium when immersed in Ringer's solution but this gain is much less than the gain in chloride. Likewise it appears that the experimental points in low concentrations of sodium lie somewhat above the 33 per cent diagonal. This divergence is greater than the experimental error and indicates either some lag in the diffusion of sodium out of the muscle or some fraction of the sodium which is more or less permanently fixed in the muscle, either because it is enclosed within impermeable cell membranes or is combined with indiffusible anions. Muscles soaked in pure

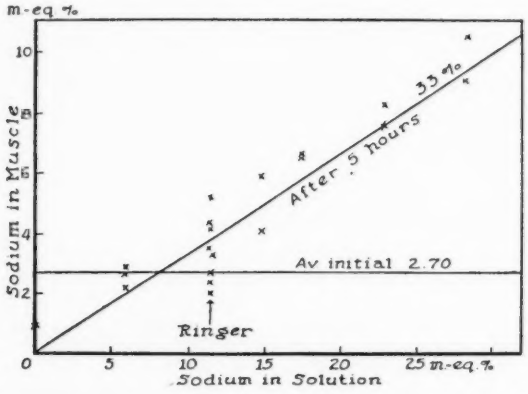

Fig. 5. Sodium content of frog sartorius muscles after soaking for 5 hours at 22°C. in solutions of different sodium content.

hypertonic sugar solutions do not lose sodium as completely as they lose chloride and the amount which remains within the muscle is roughly equal to the amount of excess sodium (1 m.-eq. per 100 grams) which is found in normal muscle (see fig. 2).

Another series of experiments was performed in which muscles were immersed in solutions of varying potassium content for 5 hours at 22°C. and were subsequently analyzed for sodium, control muscles being analyzed before immersion. The results are plotted in figure 6. In potassium-free solutions there is a gain of sodium which agrees with the small gain in Ringer's solution indicated in figure 5. As the potassium content of the solution increases the sodium content of the solution decreases and this results in a corresponding decrease in the sodium content of the muscle. Again, however, in isotonic potassium solution containing no sodium there

remains a small amount of sodium of the same general order of magnitude as the excess sodium, that is, about 0.7 m.-eq. per cent.

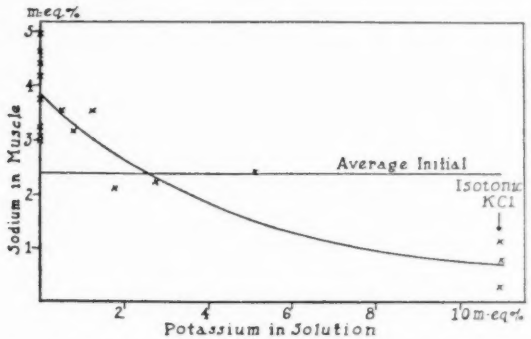

Fig. 6. Sodium contents of frog sartorius muscles after immersion for 5 hours at 22°C. in Ringer's solutions in which increasing amounts of sodium were replaced by potassium. Calculations based on weight after soaking.

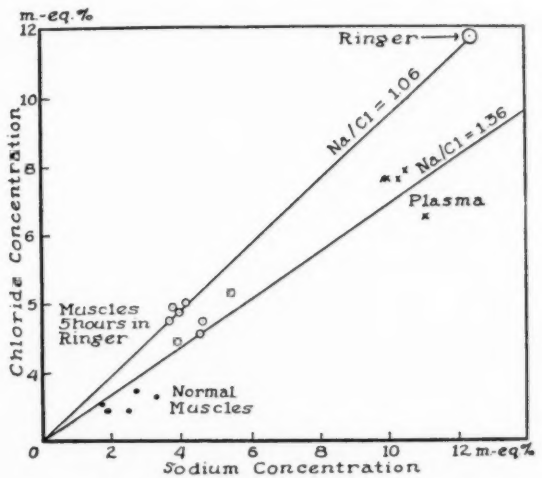

Fig. 7. Sodium and chloride contents of plasma and muscle before and after soaking in Ringer's solution. See table 2.

From the above experiments it may be concluded that there exists in normal muscles a certain amount of excess sodium which is relatively firmly fixed within the muscle in some manner and is not freely diffusible like the rest of the sodium and chloride. From figures 4 and 5, however,

it appears that immersion of muscles in Ringer's solution tends to eliminate this excess sodium, for after this treatment 31 to 33 per cent of the muscle is found to contain sodium and chloride in the same concentrations as in plasma. In order to confirm this conclusion more definitely by analyses of soaked and unsoaked muscles of the same animal, a series of five frogs was studied. The frogs were first bled and the sodium and chloride contents of the plasma were subsequently determined. Sartorius muscles (also two semitendinosus and one tibialis) were removed and soaked for 5

TABLE 2

Changes in weight and in sodium and chloride content of muscles after immersion for 5 hours in Ringer-phosphate solution

(M.-eq. per 100 grams)

FROG NUMBER	PLASMA		MUSCLE				GAIN OR LOSS OF WEIGHT
			Initial		Final		
	Na	Cl	Na	Cl	Na	Cl	
							per cent
1	8.08	4.36		0.28	4.01	2.92	−12.2
2	10.33	7.60	3.38	1.31	5.40	4.35	−3.0
3	11.1	6.56	1.89	0.93	3.89	2.89	−2.6
4	10.0	7.60	1.71	1.10	4.57	3.49	−7.8
4*	10.0	7.60	1.71	1.10	3.76	3.48	−2.3
5	10.48	7.94	2.48	0.93	3.98	3.79	+8.2
5*	10.48	7.94	2.48	0.93	3.79	3.84	+3.4
6	9.94	7.64	2.67	1.45	4.55	3.10	−2.7
6*	9.94	7.64	2.67	1.45	4.16	4.00	+2.9
Average Na/Cl...	1.47		2.14		1.21		
Average concentration in per cent of that outside†			23.5	13.8	34.2	30.3	

* Semitendinosus muscles. Otherwise sartorius muscles.

†The concentrations outside were those found in plasma for initial values and those in Ringer's solution for final values. The Ringer contained 11.69 m.-eq. per cent chloride and 12.4 m.-eq. per cent sodium.

hours in Ringer's solution before analysis, while control muscles from the upper leg were analyzed immediately for sodium and chloride as controls. The results are shown diagrammatically in figure 7, in which chloride concentrations are plotted against sodium concentrations. The plasma points are indicated by crosses and the diagonal line representing the average ratio 1.36 is drawn through this cluster of points. It is evident that the normal muscles fall below this diagonal having higher sodium to chloride ratios. All points having the same sodium to chloride ratio as a Ringer's solution (Na/Cl = 1.06) are represented by another diagonal and it is

evident that the concentrations after soaking in Ringer's solution tend to lie along this diagonal for Ringer's solution rather than along that for plasma. These soaked muscles have therefore gained both sodium and chloride during their immersion. Actually they have gained, however, on the average 1.3 times as much chloride as sodium so that the sodium to chloride ratio has gone down from 2.14, the average initial value, to 1.21. Four of the nine muscles studied have lost practically all their excess sodium (points on Ringer diagonal, fig. 7) while the other five have lost only a part of it.

Since figure 7 does not indicate which muscle points belong with which plasma points we have included the data in table 2 together with figures for the percent gain or loss in weight. There seems to be no regularity between different muscles in this respect. In a larger series of 24 muscles (13 sartorius, 6 semitendinosus, 2 tibialis and 2 ileofibularis) the average weight change was a 1.6 per cent loss; seven of the muscles gained on the average 5.3 per cent and fifteen lost on the average 5 per cent. The maximum change was a gain of 8 per cent in one muscle and a loss of 12 per cent in another. One might expect that muscles which gained in weight would show also the highest gain in sodium and chloride but it is difficult to discover any such correlation in the figures of table 2.

From the data of table 2 we have calculated the volume of the sodium and chloride spaces, both before and after soaking, in per cent of the total muscle weight (i.e., the concentration inside in per cent of that outside in the plasma or the solution). The average values so obtained for the nine muscles are given in the last line of table 2 and confirm very well the figures already given from other data on larger numbers of muscles (cf. figs. 3, 4 and 5).

Discussion. These experiments show that part of the sodium content of normal frog muscles is in excess of the chloride content and is relatively indiffusible like the potassium. It is perhaps contained within the muscle fibres and cannot penetrate the membrane. If sodium could penetrate it would of course exchange with potassium to which the membrane is believed to be permeable. There seems to be no good reason for excluding the sodium entirely from the interior of the fibres and assuming as Mond and Netter did, that this excess sodium is combined in the surfaces of the fibres in some way. One might of course assume that the fibres are impermeable to both potassium and sodium but this is not consistent with the greater mobility of potassium compared to anions as evidenced by the marked electronegativity produced by the application of potassium solutions to muscles, and it seems necessary to assume complete impermeability to either anions or cations in order to explain the retention of potassium inside the muscles.

On soaking the muscle in Ringer the chloride increases until the ratio

of sodium to chloride becomes nearly equal to the ratio in Ringer's solution. This change must bear some important relation to other changes which occur in a muscle immersed in Ringer. It is known that such muscles lose potassium and phosphate and show a progressive decrease in metabolic rate. Mond and Netter have sought to establish some connection between this excess sodium and the metabolic processes of recovery but we believe that more evidence on this point is needed. We postpone further speculation until our study of the ion exchanges between muscle and Ringer's solution is completed.

SUMMARY

1. The normal sodium to chloride ratio in frog plasma is 1.36, the chloride being subject to greater fluctuations than the sodium under different experimental conditions.

2. The normal average sodium to chloride ratio in frog muscles is 2.27 but approaches 1.06 on soaking in Ringer's solution for 5 hours at 22°C.

3. When muscles are soaked in Ringer's solution the sodium concentration increases from 24.5 per cent to 33 per cent of that outside while the chloride concentration increases from 14.5 per cent to 31 per cent of that outside.

4. In normal muscle *in vivo* 41 per cent of the sodium is in excess of the chloride; it is relatively less diffusible than the chloride and is perhaps present inside the fibres.

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THE EFFECT OF LOW ATMOSPHERIC PRESSURE ON THE GLYCOGEN CONTENT OF THE RAT

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In the course of a study of the importance of glycogen in cardiac contraction during anoxemia it was planned to follow the changes of carbohydrate in intact animals during acclimatization to low oxygen tension. For this purpose albino rats were kept in air at one-half atmospheric pressure for periods varying from one day to three months, at the end of which times they were removed and determinations made of the glycogen in the heart, gastrocnemius and liver, and of blood sugar and blood lactic acid. It soon became apparent that the most striking changes occurred during the first 24 hours at the low oxygen tension, the results suggesting that there had been in this time an absolute increase in the total amount of carbohydrate, and it is in demonstration of the truth of this suggestion that the present experiments are presented.

METHOD. Albino rats of both sexes weighing from 150 to 200 grams were used. Care was taken to feed a uniform diet regularly and to remove animals for fasting at a uniform time of day.

When samples were to be obtained the animals were anesthetized by intraperitoneal injection of nembutal (5 mgm./100 gm. body wt.). The gastrocnemius was first removed, care being taken to leave the blood supply intact until the last. The chest was then opened and the heart removed rapidly, so that less than 5 seconds elapsed between the time of cutting the chest and the immersion of the heart in KOH solution; this speed is essential in obtaining consistent values for cardiac glycogen, as will be reported more particularly in another paper. A 1 to 1.5 gram sample of liver was then taken without delay.

The method of determining glycogen was essentially the cold KOH method of Cori (1932), the final estimation of glucose being made using Somogyi's reagent no. 2 (unpublished) as described by Peters and van Slyke (1932). Checks of the cold KOH method on 1 to 2 gram samples of fresh tissue agreed within 2 per cent with determinations made by the use of the more traditional boiling 60 per cent KOH.

In the experiments in which total body glycogen was determined, the

animal was first anesthetized, and the whole liver was removed, immersed in cold KOH in a tared vessel, and chopped with scissors. The vessel and scissors were then reweighed, and the tissue then digested by heating and determined for glycogen in the usual way. In the meantime the liverless body had been completely immersed in a slush consisting of ether and CO_2 snow in which mixture it rapidly froze; it was then cut into thin slices with a tobacco slicer, digested in KOH and estimated for glycogen as indicated above.

For the heart and gastrocnemius the non-fermentable reducing substance in the final hydrolysate was found to be small and consistent but for livers and the whole body it was considerable. For the whole livers and for the bodies the non-fermentable reducing substance of the final hydrolysate was determined and subtracted from the total reducing substance found, before calculating back to glycogen content. The fermentation was done in duplicate with yeast whose activity was frequently tested.

Blood sugars were done by Benedict's method, and lactic acid following the directions of Friedmann and Kendall (1929) using the modified apparatus of West (1931). The variation expressed for all values is the standard deviation of the mean.

The chamber used for subjecting animals to ½ atmospheric pressure consisted of a 10 inch bell jar with two openings, resting on a greased ground glass plate. A wire-mesh platform was provided for the rats. From the upper opening connection was made to a vacuum pump and by separate tubing to a large mercury manometer. The inlet of air was through a tube fitted to the lower opening of the bell-jar and was so arranged that a lever actuated at one end by a float in the open arm of the manometer would alternately close and open the constricted free end of the inlet tube as the mercury rose and fell in the manometer. In this way, the position of the lever being appropriately set, the pressure in the chamber could be maintained at ½ atmosphere for as long as desired with but 1 to 2 mm. fluctuation. The ventilation through the chamber was in excess of 6 liters a minute.

The adrenalectomized animals used in these experiments were bilaterally operated at one sitting and maintained subsequently either by daily injection of 1 cc. of a commercial adrenal cortical extract or by offering as drinking water the solution suggested by Rubin and Krick (1933) and which contains the chlorides of Na, K, Ca and Mg. In order that the animals drink freely of this mixture, it is necessary to provide a dry diet. For the sake of uniformity the same dry diet was given to the animals maintained with cortical extract. No difference in the carbohydrate values, as eventually determined, was observed for the animals maintained in these two ways. With either method the most of the animals remained active, gained weight and lived beyond the expected period of survival of

untreated animals. A test group of 6 operated animals maintained on sait were all alive and well at 2 months post-operative.

EXPERIMENTAL RESULTS. Table 1 gives the results and is explanatory of the first two groups of experiments.

Attention is drawn to a number of points: First, to dispose of the results not particularly relevant to the present discussion, it may be said that low cardiac glycogen has been a consistent finding for fed animals (Long and

TABLE 1

DESCRIPTION OF EXPERIMENT	NUMBER OF ANIMALS	GLYCOGEN			BLOOD	
		Heart	Gastrocnemius	Liver	Glucose	Lactic acid
		mgm. per 100 grams	*mgm. per 100 grams*	*mgm. per 100 grams*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
1. *Intact animals:*						
a. Fed	6	414±32	643±15	2079±283	106±6	
b. Fasted 24 hours at 1 atmosphere (controls)	13	673±21	521±11	188±38	84±3	15±1
c. Fasted 24 hours at ½ atmosphere	6	867±56	806±41	2501±75	125±6	
d. Fasted 24 hours at 1 atmosphere + 3 hours at ½ atmosphere	4	657	546	146	78	17±1
e. Fasted 24 hours at 1 atmosphere + 12 hours at ½ atmosphere	4	729	623	880	95	
f. Fasted 24 hours at 1 atmosphere + 24 hours at ½ atmosphere	8	668±14	644±30	1416±87	125±6	16±1
2. *Adrenalectomized animals* (average 2 weeks post-operative):						
a. Fed	10	325±17	594±34	2252±340	94±5	
b. Fasted 24 hours at 1 atmosphere	8	391±38	484±4	143±9	60±3	
c. Fasted 24 hours at ½ atmosphere	11	476±21	559±14	149±20	68±5	
f. Fasted 24 hours at 1 atmosphere + 24 hours at ½ atmosphere	11	388±16	501±19	131±6	62±4	
g. Right adrenal removed. Left adrenal intact but splanchnic nerve cut. Fasted 24 hours at 1 atmosphere + 24 hours at ½ atmosphere	4	665	720	1251	98	

Evans (1932)), and for adrenalectomized animals (see expts. 1a and 2a–f). The values for fed animals have been included because it was desired to emphasize that not only did the adrenalectomized animals gain weight and appear healthy, but that they had, when fed, approximately the same carbohydrate stores as fed normal animals (1a and 2a) and therefore the failure to obtain the same results for adrenalectomized as for normal animals after subjection to low oxygen tension can not readily be laid to general malnutrition through failure of appetite and digestion.

More pertinent, it will be seen that intact animals placed to fast for 24 hours at ½ atmosphere (1c) do not lose their liver glycogen store as do ordinarily fasted animals (1b), but indeed appear to have a liver glycogen content somewhat greater than was found for fully fed animals; and the increased values for heart and gastrocnemius glycogen and for blood sugar make it improbable that this liver glycogen has been derived from carbohydrate elsewhere in the body.

When the same comparison is made for adrenalectomized animals (2b and c) it is seen that the evidence for increase or maintenance of liver glycogen during exposure to low oxygen tension is entirely lacking, although there is a suggestion that the other carbohydrate stores have been moderately increased.

So far it might be argued that the maintenance of liver glycogen of intact animals during fasting at ½ atmosphere had been due to failure of breakdown to occur and that no necessity existed to hypothecate new formation. But when liver glycogen was lowered by a preliminary fast

TABLE 2

DESCRIPTION OF EXPERIMENT	NUMBER OF ANIMALS	GLYCOGEN PER 100 GM. BODY WEIGHT		
		In liver	In rest of body	Total
		mgm.	*mgm.*	*mgm.*
Fasted 24 hours at 1 atmosphere	10	9.6±1.3	225±9	235±9
Fasted 48 hours at 1 atmosphere	12	12.4±1.6	203±6	215±7
Fasted 24 hours at 1 atmosphere + 24 hours at ½ atmosphere	10	59.0±6.4	230±11	288±17

at atmospheric pressure it was found to be largely replaced during a further fast at ½ atmosphere (1f), without the occurrence of any lowering in the other carbohydrate stores to explain the origin of this new liver glycogen. Here again the adrenalectomized animals did not exhibit the same phenomenon (2f). It is especially to be observed that animals with one adrenal removed and the other denervated (2g) behave as normal animals when exposed to low pressure.

To explain an increase of liver glycogen during fasting one naturally thinks of the possibility of epinephrine action. That no great discharge of epinephrine occurs in these experiments is indicated by the fact that three hours after being placed in ½ atmosphere (1d) the fasted animals have practically the same carbohydrate values as when first put in, whereas if there had been any appreciable discharge of epinephrine coincident with the onset of low oxygen tension we should have expected (Cori and Cori, 1928b) by this time a decrease in muscle glycogen and an increase in liver glycogen with some disturbance of blood sugar and blood lactic acid.

The values after 12 hours in the chamber (1e) are intermediate between those at 3 and at 24 hours.

It will be observed that the blood lactic acid values, being the same in the three groups examined, offer no clue as to the mechanism.

The third group of experiments in which the total glycogen of intact animals was determined is represented in table 2. It will be observed that animals fasted 48 hours have less glycogen than after a 24 hour fast, this finding agreeing with that of Cori and Cori (1928a) and being no doubt explained by the loss of glycogen in skeletal muscle (Long and Evans, 1932).

But 48 hour fasted animals which have been subjected to ½ atmosphere for the second 24 hours contain 34 per cent more glycogen than animals fasted 48 hours in room air, the increase being chiefly due to new-formed liver glycogen.

Experiments to determine the source of this extra glycogen are now in hand.

CONCLUSIONS

It appears then that there is a substantial increase in the total carbohydrate of rats when they are placed without food for 24 hours at ½ atmosphere. The greatest increase occurs in the liver and can not be accounted for by decreases in carbohydrate elsewhere, as would be the case if we supposed this phenomenon to be due to any hitherto accepted action of epinephrine. All recognized forms of carbohydrate occurring in the body in amounts capable of accounting for such a large increase are themselves found increased or undiminished.

Under these circumstances it seems reasonable to assume that we have been observing an *interconversion of either protein or fat to carbohydrate* in excess of oxidative needs. It is indicated that this conversion is dependent on some adrenal tissue being present.

I wish to thank Dr. C. N. H. Long for his interest during the course of this work.

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PLATELETS AND THE SPONTANEOUS SYNERESIS OF BLOOD CLOTS

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There is some conflict of opinion over the rôle of the platelets in the syneresis of blood clots. Some observers (1) (2) (3) (4) (5) (6) affirm that syneresis is strictly a function of the platelet, while others (7) (8) (9) (10) (11) point out that it is a property exhibited by many gels and that the platelet plays only a secondary part in it. Much of this disagreement may be traced to confusion resulting from different methods of counting platelets and of estimating syneresis, and the use of this term to designate both the spontaneous retraction of a clot and that following the artificial separation of the gel from the sides of the vessel. It has been abundantly demonstrated that clots from deplateletized blood or recalcified oxalate plasma, free of platelets, will retract even in narrow glass vessels if the gel is gently loosened from the walls (8) (10) (11). Since it has not been denied, however, that platelets improve the degree of syneresis, that undisturbed clots from deplateletized blood or plasma held in narrow glass tubes contract very slowly or not at all, it seems probable that the function of the platelets consists in overcoming forces which, under certain conditions, would keep a clot from retracting. Adhesion may prevent or delay the retraction of many gels. The evidence presented here indicates that, when by virtue of prevailing conditions, this force is in dominance, clots from the blood of the dog or man do not retract; they do so if intact blood platelets, even from another species, are present in adequate numbers.

METHODS. All observations, unless otherwise stated, were made on human and dog blood or recalcified oxalate plasma poured in amounts of 1 cc. into glass tubes 8 mm. internal diameter and kept at 37°C. (±1°). These conditions were adhered to in all comparative experiments because it was found in preliminary observations, to be described later, that aside from changes in the blood itself, the nature of the containing vessel and the temperature, syneresis may be influenced by the extent of the free surface of the clot and the ratio between it and the extent of the covered surface. One cubic centimeter of blood that has clotted in a tube of the type just described offers a free surface of approximately 50 mm.[2] and a

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surface exposed to adhesion of approximately 448 mm.^{2} or a nearly 1–9 ratio of free to covered surface. Under these conditions deplateletized blood or ordinary recalcified oxalate plasma freed of platelets by prolonged centrifugalization does not, upon clotting, show any spontaneous syneresis for 48 hours. Such clots will be arbitrarily referred to as *irretractile* and form the basis of most of the experiments here presented. Oxalate plasmas were recalcified by adding to them the optimum amount of 0.5 per cent $CaCl_2$ previously determined for that particular sample. Immediately after recalcification the plasma was distributed to the vessels. Tubes were flamed for a few minutes and cooled before using; to prevent evaporation, they were corked. Platelets were isolated by fractional centrifugalization of 50 to 75 cc. of blood in sterile, cold 3 per cent citrate saline solution, washed twice, suspended in 0.8 per cent salt solution and used on the same day that they were collected. Platelet counts were made by the direct method using as a diluent 3.6 per cent sodium citrate and 0.05 per cent Brilliant Cresyl Blue. Duplicate determinations, preliminary filtering and chilling of solutions, the use of chilled, certified pipettes and automatic shaking were precautions taken with each count. The total amount of syneresis in 48 hours was arbitrarily recorded in units and these were determined by *adding* the figures representing in tenths of 1 cc. the approximate amount of serum expressed at definite intervals after clotting took place: thus if the clot at the end of one-half hour had expressed 0.2 of its volume in serum; and at the end of one hour, 0.3; of two hours, 0.5; and so remained at the end of 24 and 48 hours, the total number of units of syneresis for that clot would be 2.0. The observations were always made at the intervals of time just noted. The units of syneresis for a normal blood clot are usually 2–3, but for plasma they may go as high as 4. Since the clot was not to be disturbed, these figures were arrived at approximately, by measuring the clot from the outside of the tube in three directions, calculating its volume from these figures and subtracting that from 1 cc., thus obtaining in tenths of 1 cc. the amount of expressed serum. In preliminary trials of the method the figures were found to correspond very closely to the actual amount of serum expressed.

The anti-platelet serum was prepared by giving to a rabbit at 5 day intervals six intravenous injections of the washed platelets from 100 to 120 cc. of blood. The resulting anti-serum is species specific (12); so that human and dog platelets, respectively, were used in the making of anti-human platelet serum and anti-dog platelet serum.

TERMINOLOGY. *Syneresis.* The spontaneous retraction of an undisturbed clot within 48 hours after it is formed.

Deplateletized blood. Blood from dog or man with a platelet count not over 20,000 per c.mm.

Intact platelets. Platelets that have not been modified by clumping,

disintegration or by coming into contact with toxic substances, and that have been used not later than 12 hours after collection under proper precautions.

RESULTS. *Syneresis and the extent of the free and covered surfaces of the clot.* The degree to which a clot will retract may be influenced by: a. The extent of the free surface of the clot. The syneresis of platelet-free plasma varies directly as the free surface. Uniform amounts (3 cc.) of recalcified oxalate plasma, free of platelets, were placed in glass vessels measuring respectively 5, 8, 17, 25 and 50 mm. in diameter. Syneresis was greatest in the last two vessels, poor in the third, and absent in the first two. With platelet-rich plasma, syneresis appeared in all vessels, but it was greatest in the first two. b. When the area of the surface of a clot exposed to adhesion is about equal to that of its free surface, syneresis may appear even in narrow glass tubes and in the absence of platelets. Platelet-free plasma was placed in glass tubes 8 mm. in diameter in varying amounts so as to yield the following ratios between the calculated free and covered surface areas: 1–20, 1–10, 1–5, 1–2½, 1–2, 1–1. Retraction was moderate in the last one, slight in the preceding one, and absent in the others. Clots from platelet-rich plasma handled likewise also retracted to a greater degree when their free and covered surfaces were about equal in extent. For these reasons it appears essential that these factors be taken into account in all comparative experiments on spontaneous syneresis.

Evidence pointing to the platelet as playing a part in clot retraction. a. The addition of dog platelets in adequate amounts induces syneresis in irretractile platelet-free dog plasma.

Platelets suspended in 0.1 cc. of 0.8 per cent salt solution.
1 cc. plasma → clot in 6½ minutes = 0 unit of syneresis.
1 cc. plasma + 0.1 cc. of 0.8 per cent salt solution → clot in 6 minutes = 0 unit of syneresis.
1 cc. plasma + 20 million platelets → clot in 1½ minutes = 0 unit of syneresis.
1 cc. plasma + 200 million platelets → clot in 1½ minutes = 0.5 unit of syneresis.
1 cc. plasma + 600 million platelets → clot in 45 seconds = 1.6 unit of syneresis.
1 cc. plasma + 3 billion platelets → clot in 30 seconds = 0.8 unit of syneresis.
1 cc. plasma + 8 billion platelets → clot in 1 minute = 0.2 unit of syneresis.

These experiments were repeated, with essentially the same results, with deplateletized blood from dogs (after administration of anti-platelet serum) and with deplateletized human blood. For deplateletized dog's blood, the best syneresis was obtained after addition of 500 million to a billion platelets per cubic centimeter of blood. With deplateletized human blood, syneresis was poor when 150 million or less platelets per cubic centimeter of blood were added, and high when from 300 to 800 million platelets were used. Platelets added to deplateletized blood after clotting did not restore syneresis even when introduced into the substance of the clot.

b. The location of the platelet suspension and spontaneous syneresis. Recalcified oxalate dog plasma was placed in a U-shaped glass tube measuring 5 mm. in diameter and 10 cm. in length, and at one extremity of the column of the plasma 1 drop of a well emulsified suspension of fresh platelets was added. After clotting, syneresis was immediate and strong about the location of the platelets, and absent in the other extremity of the tube after 12 hours. Into a glass trough 1 mm. deep, 4 cm. long, and 3 mm. wide, a thin film of recalcified oxalate dog plasma was poured; at one end of the trough a drop of a suspension of fresh platelets was carefully placed. After clotting, there was immediate strong syneresis of the film at the end of the trough where the platelet suspension was placed and none was detected at the other end after 12 hours.

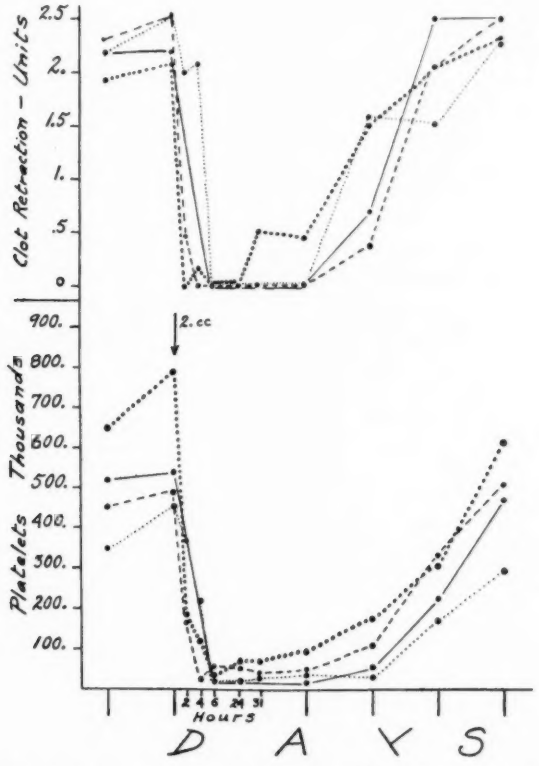

Fig. 1. Effect of the intraperitoneal administration of anti-platelet serum in 4 dogs.

c. In figure 1 are shown the changes in the platelets and units of syneresis at various intervals following the intraperitoneal injection of 2 cc. of anti-platelet serum into 4 dogs. The degree of syneresis is shown to be related to the platelet level of the blood, since its disappearance accompanies a drop in platelets, and its reappearance follows a rise in their number. When anti-platelet serum was repeatedly given intravenously to one animal, similar changes were observed. (Fig. 2.) Normal rabbit serum intraperitoneally or intravenously did not bring about any similar changes.

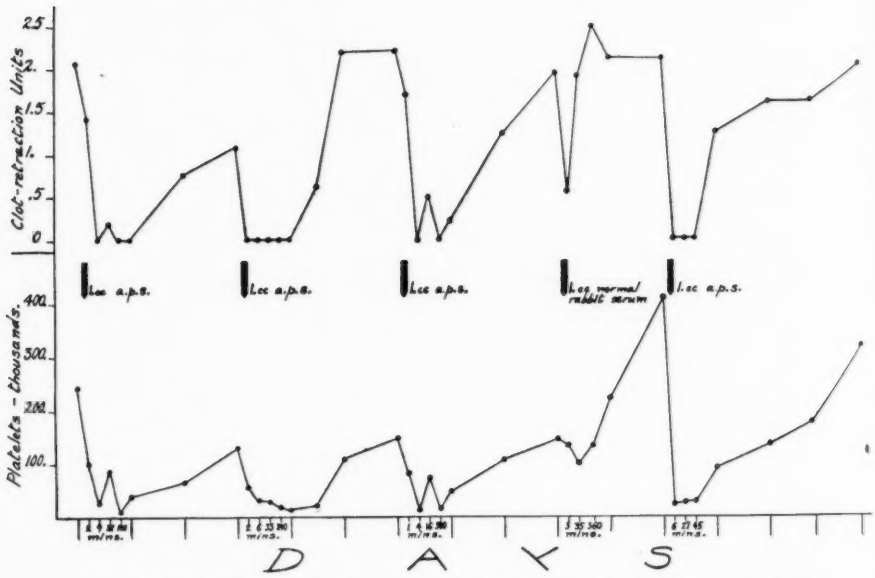

Fig. 2. Effect of the repeated intravenous administration of anti-platelet serum and of one dose of rabbit serum in one dog.

Intact and destroyed platelets. The absolute number of platelets in any determined amount of blood may not give an indication of the degree of syneresis of its clot, as any evidence of their action depends on the number of *intact* platelets left after clotting. Eberth and Schimmelbush (13), LeSourd and Pagniez (2), Aynaud (14), and Lee and Vincent (15), have observed that not all platelets are destroyed during the process of coagulation. An identical conclusion can be reached by observing under the microscope the changes in a drop of platelet-rich human oxalate plasma undergoing coagulation. The platelets at the edge of the drop undergo clumping and disintegration very soon after the preparation is made, fibrin needles shoot across the plasma and within 10 minutes the slide can be

moved about without the drop changing its position, indicating that coagulation has been completed. For as long as 24 hours afterwards, many isolated platelets, which have kept their shape, can be observed, particularly in the center of the preparation. Essentially similar observations can be made with platelet-rich plasma from hemophilic humans. If a drop of anti-platelet serum is added to that plasma, however, nearly every platelet in the preparation appears agglutinated and disintegrated in a few minutes. It had been observed before that, when the proper amount of anti-platelet serum was added to hemophilic or normal blood, syneresis was completely inhibited. It seemed likely, therefore, that blood with an adequate number of platelets may yield an irretractile clot if all or most of its platelets were destroyed during the process of coagulation. Conversely, the strong syneresis of some blood clots (the hemophilic, for example) might then be due to the great number of intact platelets left after coagulation had occurred.

Human and dog platelets were isolated and suspended in a known concentration, in salt solution. Various definite amounts of destroyed and undestroyed platelets were added to irretractile deplateletized human and dog's blood respectively. The platelets were destroyed by shaking them in a paraffined tube holding a quantity of distilled water corresponding to approximately 1,000 times their total volume, and placing them in an incubator at 37°C. for 2 hours. The distilled water was centrifuged off after that interval and the residue suspended in 0.1 cc. of 0.8 per cent salt solution.

R. S., 23, female. Platelets: 16,000 per c.mm.

1 cc. blood + 180 million intact platelets → clot in 6½ minutes = 1.7 unit of syneresis.

1 cc. blood + 180 million destroyed platelets → clot in 5¼ minutes = 0 unit of syneresis.

1 cc. blood + 90 million intact platelets → clot in 6½ minutes = 0.8 unit of syneresis.

1 cc. blood + 90 million destroyed platelets → clot in 7½ minutes = 0 unit of syneresis.

1 cc. blood + 0.1 cc. of 0.8 per cent salt solution → clot in 18 minutes = 0 unit of syneresis.

1 cc. blood → clot in 17 minutes = 0 unit of syneresis.

In one series of experiments platelets collected from hemophilic individuals were used; no difference in their behavior was detected from that of similarly treated platelets from normal humans. With deplateletized dog's blood similar results were observed, but a higher number of intact platelets had to be added to bring about syneresis (at least 150 million per cubic centimeter of blood).

Further evidence in support of the view that the proportion of undestroyed platelets influenced syneresis:

H. K., 22, male. Normal human adult. Platelets: 234,000 per c.mm.
1 cc. blood + 0.1 cc. of anti-normal human platelet serum → clot in 5 minutes = 0 unit of syneresis.
1 cc. blood + 0.1 cc. of 1–2 dilution of anti-platelet serum → clot in 5 minutes = 0.7 unit of syneresis.
1 cc. blood + 0.1 cc. of 1–4 dilution of anti-platelet serum → clot in 8 minutes = 1.2 unit of syneresis.
1 cc. blood + 0.1 cc. of a 1–8 dilution of anti-platelet serum → clot in 7 minutes = 1.8 unit of syneresis.
1 cc. blood + 0.1 cc. of 0.8 per cent salt solution → clot in 10 minutes = 1.8 unit of syneresis.
1 cc. blood → clot in 9 minutes = 1.8 unit of syneresis.

Similar results were obtained with normal dog's blood. With human blood of higher platelet content (900,000 or more per c.mm.) 0.2 and even 0.3 cc. of anti-platelet serum was necessary to completely inhibit syneresis of 1 cc. of blood. The addition of anti-platelet serum to deplateletized blood or to platelet-free recalcified oxalate plasma did not alter their irretractility and did not seem to change the appearance of the clot.

Howell (8) has observed that the syneresis of clotted plasmas is very marked when clotting is brought about by solutions of thrombin. That thrombin by itself cannot bring about syneresis of an irretractile clot is demonstrated by the experiment below:

Dog 9. Platelets: 360,000 per c.mm. of blood.
1 cc. blood + 0.1 cc. of fresh dog's serum (½ hour old) → clot in 30 seconds = 2.2 units of syneresis.
1 cc. blood + 0.1 cc. of dog's serum (4 months old) → clot in 7½ minutes = 2.1 units of syneresis.
1 cc. blood → clot in 6 minutes = 1.3 unit of syneresis.

Dog 32. Platelets: 14,000 per c.mm.
1 cc. blood + 0.1 cc. of fresh dog's serum (½ hour old) → clot in 50 seconds = 0 unit of syneresis.
1 cc. blood + 0.1 cc. of dog's serum (4 months old) → clot in 3½ minutes = 0 unit of syneresis.
1 cc. blood → clot in 4 minutes = 0 unit of syneresis.

Thrombin was present in the fresh sera in sufficient concentration to accelerate the clotting of the thrombopenic blood, but it was incapable of restoring syneresis to it. When the fresh serum was diluted twice or four times, or added to the blood in slightly higher quantities, there were no significant changes from the examples given above. Serum a few days old likewise did not restore the retractility of deplateletized blood. It is likely, therefore, that the greater syneresis of normal blood brought about by thrombin is a result of a platelet-sparing action of that substance. Because clotting takes place more rapidly, not many platelets are destroyed, and a greater number of them are available for syneresis. Clear saliva

from hemophilic and normal human individuals when added to hemophilic and normal blood had a clot accelerating and syneresis exalting action similar to that of thrombin; when added to deplateletized blood, clotting was accelerated but syneresis was not restored.

Modified platelets. Ageing of the platelets reduced their ability to bring about syneresis. After three days at 5°C., they had lost this property. Placing them for one hour in a 1–10,000 solution of saponin, and washing the residue in isotonic salt solution likewise inactivated them. Drying in a watch glass or shaking them violently for one-half hour led to similar results. Platelets heated to 60°C. for one-half hour did not restore syneresis to deplateletized dog's blood; however, their addition in moderate numbers to normal blood did not alter syneresis.

Addition of platelets from one animal species to blood from another. The property of inducing syneresis possessed by the blood platelets is not species specific. Suspensions of platelets from the dog and rabbit were added in different amounts to irretractile human blood; rabbit and human platelets were added to irretractile dog's blood. In every instance syneresis was restored to the clots in a degree directly proportional to the number of platelets added, within the limits already stated.

Discussion. As stated from the beginning, all comparative observations were made on clots that were not artificially separated from the sides of a narrow glass vessel. In some instances, as a trial, they were gently separated and invariably some serum was expressed even from the deplateletized blood clots. This fact, besides other accumulated evidence, indicates that mammalian blood or plasma, even when free of platelets, possesses all the elements needed for syneresis. Its promptness, degree, or any manifestation at all of its presence under certain conditions, are greatly helped by an adequate number of intact blood platelets. It is conceivable that at times certain plasmas may acquire unusual properties of retractility, and may then exhibit syneresis under adverse conditions. The irretractility of clots of blood from patients with thrombopenic purpura may be due, on the other hand, as much to changes in plasma constituents as to the thrombopenia. Howell (8) has demonstrated that fibrinogen solutions can be so altered as to yield a structureless, irretractile clot and LeSourd et Pagniez (2) have found that platelets in adequate numbers are powerless to restore syneresis to a plasma, the elements of which have been modified by one of several agents.

Just how the platelet helps to bring about syneresis is not clear. That it does so by increasing the viscosity of the blood, as suggested by Pickering and Hewitt (10) is unlikely, as the addition of modified platelets in adequate numbers does not restore the syneresis and intact platelets in excessive numbers retard it. It is possible, as Howell (8) has suggested, that platelets may be connected with clot retraction in that, as a source of

thrombin and thromboplastic substance, they increase the firmness of the clot. Yet, blood containing a large number of destroyed platelets and deplateletized blood to which thrombin has been added would conceivably have these substances in high concentration and not show syneresis. A series of experiments along this line led Arthus and Chapiro (3) to the conviction that when platelets act as agents for clot retraction, they do so like living elements. Analogous conditions are necessary for certain functions of other cells. Motility and phagocytosis are properties of leukocytes that likewise cease upon death of the cell. Efforts to extract from the platelets a principle that would induce syneresis have been fruitless (2) (16). This makes it probable that when inducing syneresis the platelet acts as a physiologic unit and not through any by-products of its disintegration.

That platelets from one animal can induce syneresis in the blood clots of another from a different species is made more interesting by the fact that Vinci and Chistoni (5) noted that the blood of birds is irretractile, but that it can be made retractile by adding to it mammalian platelets. Thrombin has also been shown to be non-specific in its action towards fibrinogen (8).

SUMMARY

The platelet-free blood plasma of man and the dog possesses all the elements needed for spontaneous syneresis, but adhesion of the clot to the sides of the vessel, if extensive enough, may delay or prevent it. An adequate number of *intact* platelets improves the degree of syneresis in general, and will overcome adhesion of the clot when present. These properties appear to belong only to the unmodified blood platelet and are not species specific.

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MANIFESTATIONS OF SEGMENTATION IN MYELINATED AXONS[1,2]

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During the course of some experiments which had as their object an end which it was felt might be gained through an assessment of effects exerted by axon potentials of different known configurations, a set of curious pictures was obtained which were finally interpreted as signifying that the action potential of a single myelinated axon is composed of more or less discrete contributions from a number of successive segments. This segmental composition of the axon potential can be demonstrated, we have found, in a number of different ways but best, perhaps, by anodal polarization of the region supplying the record. Since the results obtained through polarization gave the clue to the nature of the phenomenon it is proposed to describe these first.

METHODS. To obtain single axon spikes use has been made of the sciatic-phalangeal preparation of *Rana pipiens* (formerly called the plantar preparation, Blair and Erlanger, 1933). The entire length of nerve from from spinal emergence to the dorsum of the third phalanx of the fourth digit is made available. The large central end of the nerve (see fig. 1) is stimulated, usually about once a second, with break induction shocks and the action potentials, after conduction to the toe nerve, are led through the electrodes, *P* and *D*, into the cathode ray oscillograph after amplification up to 2,000,000 times. Along the *nervus interstitialis dorsi* III of this preparation there is a branchless stretch about 5 mm. long. In a number of experiments, in order to assure ourselves that branches were not complicating the pictures, records were obtained from this stretch. This was not done regularly because the amplitude of the spikes from this thicker part of the preparation is apt to be insufficient. Cams *1* and *2*, starting and stopping the polarizing current, and *3*, operating the stimulating switch, and the sweep contact cam (not shown) all rotate on one shaft.

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One terminal of the polarizing circuit was connected effectively with the ground, or proximal lead; the other terminal was attached either proximally (with respect to the stimulating electrodes) to the ground lead (the "three-electrode arrangement") or to the grid electrode (the "two-electrode arrangement"). Figure 1 shows the arrangement of the circuit in the

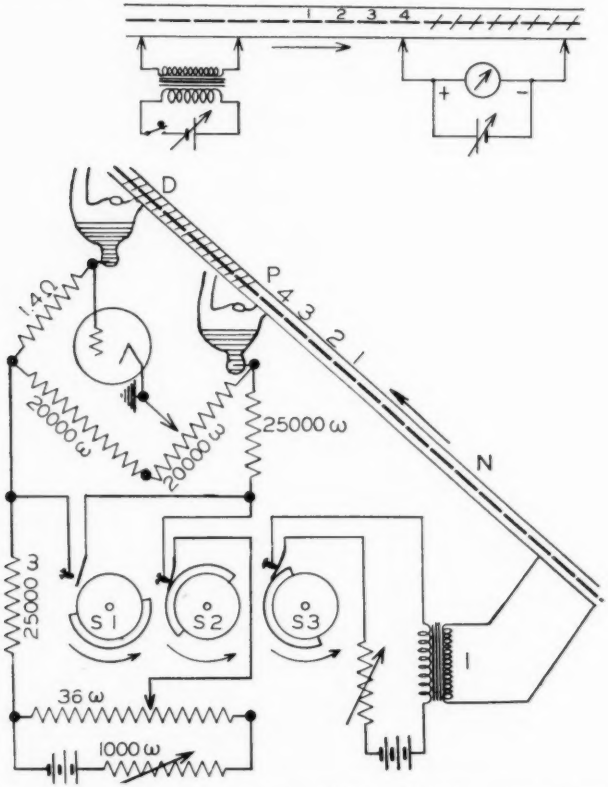

Fig. 1. Diagrams of apparatus; upper, simplified. *N* is the nerve, one myelinated axon of which is indicated by the broken line. The records obtained (see fig. 2) indicate that the part shaded was killed.

latter case. By means of a bridge balance arrangement, a modification of Bishop's (Blair and Erlanger, 1933), it was possible to polarize the nerve through the lead electrodes without disturbing the amplifier excepting during transient periods. All electrodes were nonpolarizable, of the mercury-calomel type. In some experiments the action potential was recorded during the steady portion of a momentary polarizing current; in

others the polarization was continuous. The results obtained by the two methods were essentially alike. With the two-electrode arrangement the noise level was increased somewhat and the more the stronger the

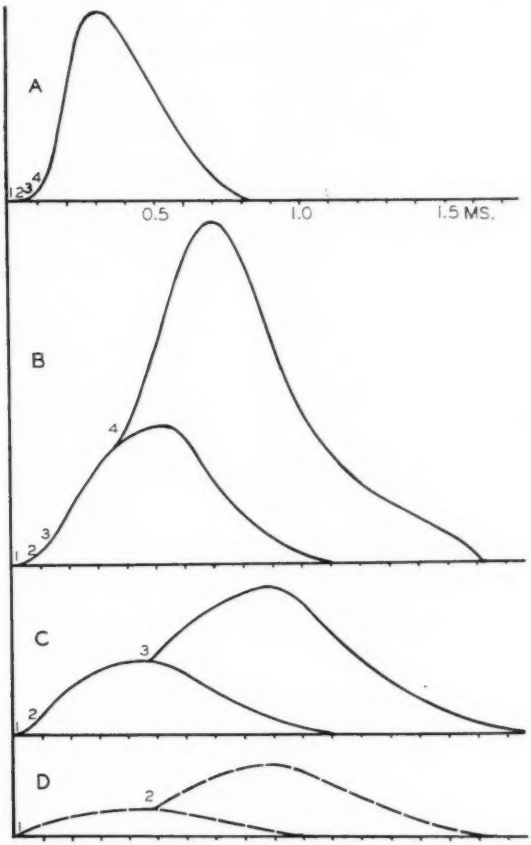

Fig. 2. Deflections obtained from the nerve diagrammed in figure 1. *A*, *B* and *C*, traced from records; *D*, seen but not recorded. *A*, normal axon potential in unpolarized nerve. *B*, *C* and *D*, three critical stages of increasing anodal polarization.

The lower curve in each case is what remains of the action potential after defection of the part above the notches, *4*, *3* and *2*, in *B*, *C* and *D*, respectively.

polarizing current. The disturbance was due to vibration of the fluid electrodes which, through the associated changes in resistance, altered the polarizing current and initiated significant transients. The pictures obtained differ markedly from preparation to preparation but they are all

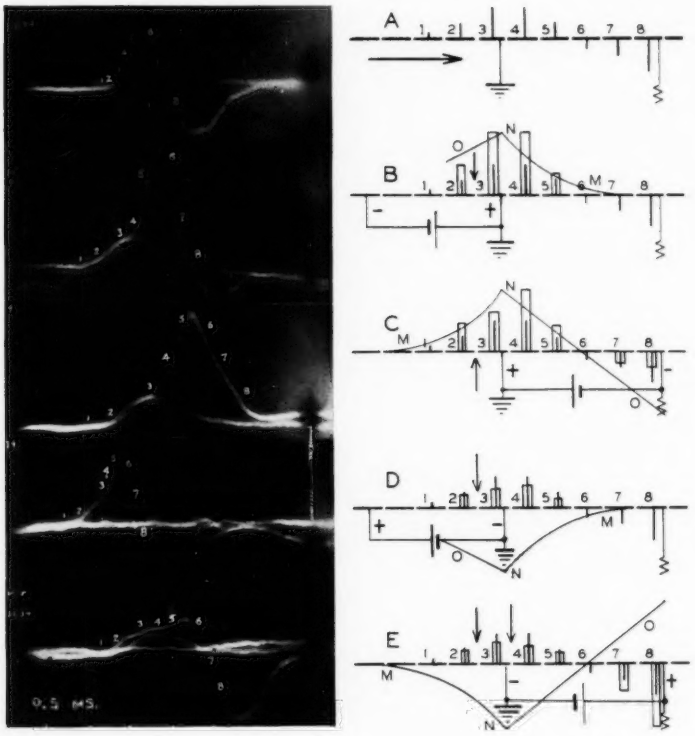

Fig. 3. Effects of anodal and cathodal polarization of the responding part of an axon. Very much reduced. The amplitudes are low because this part of the nerve is relatively thick. Some of the pictures are atypical, but this particular series is reproduced because it is the best complete series we have succeeded in obtaining from one and the same unbranched preparation. The diagrams indicate in a roughly quantitative manner our views relative to the composition of the axon potential in each case. The arrows indicate blocking positions. The possibility of contributions to the positive phase from segments beyond the distal lead is not taken into consideration.

A. The normal axon spike, with base line recorded under identical conditions. The action potential is diphasic. One can almost imagine an angle at 3.

B. Anodal polarization at the proximal lead, three electrode arrangement. Two successive sweeps under identical conditions; one shows the total response, in the other the higher wave is blocked. The records have been retouched and superimposed.

C. Anodal polarization at the proximal lead, two-electrode arrangement. There are three definite waves on the ascending limb and probably three in the descending limb and very much depressed positive phase. Two successive deflections under

of a type which we shall attempt to describe (see figs. 3, 4, 5 and 7 and legends). It should be added that this paper is concerned with polarization merely as a means to an end. Since the interpretation of our data is largely a matter of opinion our material will be presented as objectively as possible.[3]

Alterations in the configuration of the axon potential produced by polarization. 1. Anodal polarization at the ground (proximal) lead. (a) The three electrode arrangement. (See figs. 2 and 3, *B*.) As the strength of the polarizing current is increased the axon spike increases in height and duration. These are the well known effects of anodal polarization. The start of the action potential does not, however, change its position appreciably. Then, usually with a polarizing current some three to four times threshold, an angle appears in a relatively low position on the spike and mounts higher and higher as it becomes later and later until, at a critical polarization strength, all of the spike record above and later than the angle drops out together with any positive potential the record may have had, leaving behind a more or less typically shaped, strictly monophasic action potential, lower in amplitude, however, and broader than the original action potential. If the current strength is held at this critical level spontaneous changes in the nerve's state (Blair and Erlanger, 1933) cause the picture to change, but without any predictable sequence, between the complete one and the lower one (fig. 2, *B*). It therefore is possible, without altering any of the conditions, to superimpose the two pictures one on the other. The defection usually occurs when the angle is about at the crest of the underlying wave; but not infrequently the angle at this time is later than the crest, it may be by as much as 0.2 millisecond (ms.). Then the defection occurs without altering in the least either the ascending limb or the crest of the wave anterior to it (see fig. 4). With further increase in the strength of the polarizing current another series of pictures is obtained quite like the first except that the amplitudes are lower (*C* in fig. 2); and after this second defection still a third usually can

[3] Excepting where specified observations were made at room temperature, usually 20 to 23°C.

identical conditions. The block develops between waves *2* and *3*. The base lines have shifted slightly laterally with respect to each other. In other records, not so satisfactory in other respects, the unblocked deflection is definitely, though only slightly, diphasic; here a base line shift gives the impression that it is not.

D. Cathodal polarization at the proximal lead, three-electrode arrangement. Three successive sweeps, one with an action potential. Some base line shift. At this grade of polarization block developed under the anode which is central to the leads.

E. Cathodal polarization at the proximal lead, two-electrode arrangement. Three successive sweeps, one showing an unblocked response, another with a response blocked between either *2-3* or *3-4*; the third is without a response. There is some base line shifting.

be obtained (*D* in fig. 2). The amplitude of the action potential then remaining usually is but little above the noise level of the recording system under these circumstances. Therefore, the process cannot be followed, if, indeed, it continues, beyond this defection. Not infrequently, with polarization strengths just short of producing the first defection, the ascending limb exhibits two or even three notches and consequently three or four waves of which the last usually is the highest and the longest, and the first quite indistinct (see figs. 3 and 4); and similar waves may also be seen on the descending limb of the action potential. When defection occurs under these circumstances it usually is the uppermost and highest of the waves that drops out first, as in figures 2 and 7, though occasionally

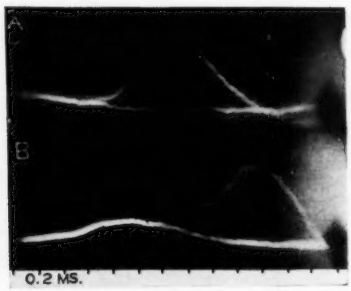

Fig. 4. Illustrating a late defection. The records are not typical.

A. Normal axon potential on its bent base line.

B. Anodal polarization at ground lead (cathode central to ground). Three successive deflections with polarization constant, one exhibiting several waves and two one wave only, all of the others being blocked out at the first notch. The first wave is superimposed on itself in all three of the deflections. The time from the start of the wave to the first notch is 0.72 ms., and the first notch is about 0.15 ms. behind the crest of the first wave.

it may be the two uppermost on the ascending limb, together, in both cases, with all subsequent waves, as in figures 3 and 4.

(b) With the *two electrode arrangement* the pictures obtained (fig. 3, *C*) differ from those described above mainly through effects produced by the cathodal polarization of the nerve under, and in the vicinity of, the grid (distal) lead, to some extent, also, as a result of a slight shift in the relative positions of the ground lead and the polarization exerted through it, and possibly also because of an effect determined by the direction the impulse is traveling relative to the direction of polarization. If the last is a factor, we have not been able to evaluate it. The amplitude at the cathode becomes so low (the usual but not the invariable effect of cathodal polarization is to lower amplitude) that accurate analysis of what is transpiring

there is impossible. It can, however, be seen, though indistinctly, that notches develop on the whole of the diphasic record spaced about as are those on the negative phase. The changes in the negative (first) phase duplicate those described above; and again the action potential becomes strictly monophasic when the uppermost negative element drops out.

2. Cathodal polarization at the ground (proximal lead). (a) *Three electrode arrangement* (fig. 3, *D*). Under these circumstances either the anode may completely block the impulse before, with increasing polarizing potential, any significant change has taken place in the picture, or one of several changes may occur: the action potential may diminish in height or it may increase, the latter apparently occurring only when the action potential is diphasic. In either case a notch or notches may appear on the ascending limb which is usually drawn out beyond the normal duration.

(b) *Two electrode arrangement* (fig. 3, *E*). This usually diminishes the amplitude of the negative phase, causes two to three indistinct wavelets (separated by notches which at this blocking stage are spaced just about as are the notches at the blocking stage of anodal polarization) to appear on it and greatly increases the depth and duration of the positive phase. The latter also may become notched. With increasing polarization the depth of the positive phase then diminishes and often in steps; if it exhibits notches successive defections may eliminate these in succession from behind forward. But not infrequently the whole of the positive phase and with it the uppermost wave on the negative phase are wiped out with the very first defection, the action potential becoming perfectly monophasic, as in figure 3, *E*.

There is in these polarization pictures much more of detail still to be presented, but the mustering of it can be facilitated by first developing a working hypothesis. For the latter purpose it is necessary to have available the results of a series of experiments in which pictures comparable with those produced by polarization were obtained through a procedure of a wholly different kind.

Segmental manifestations of salt action. In the hope of obtaining further light on the questions raised by the above observations we have investigated the action of $CaCl_2$ and KCl on the configuration of the axon potential. These salts were used because they are known to produce effects that simulate in many respects anodal and cathodal polarization, respectively (Graham, 1933).

It was a simple matter with the routine method of handling our preparations to expose the nerve on the leads to the different Ringer's solutions. It will be recalled (Blair and Erlanger, 1933) that the fine end of the nerve is mounted vertically on the lead electrodes. The latter are glass tubes and the nerve is drawn across their terminal, slit-like openings, which are about 1 mm. in width, and is held to them by surface tension. The Ringer's

solution can be made to run through the electrode, out of its pore onto and down the nerve. When this is done through the ground (the lower) electrode the solution can be made to run either some distance along the nerve toward, but not to, the stimulated end, or off of the lower edge of the electrode. In the latter case probably not more than 3 to 4 mm. of nerve on and central to the lead are exposed, and perhaps decrementally, to the action of the solution running from the electrode. When the solution is supplied through the grid (the upper) electrode it runs down the interlead stretch and on beyond the ground. The calcium and potassium concentrations were altered merely by adding more and more of their isotonic solution to the Ringer's solution that was made to run through the electrode. This was done until the desired result was obtained. The exact concentration of the salt to which the nerve was exposed at any given instant was not ascertainable. All that can be said is that higher concentrations were needed to produce effects when the solution was first applied and even then it acted slowly; once the action had been unfolded, the effects developed with lower concentrations and more quickly.

Increased calcium concentrations were found to induce alterations in the axon potential, which could be made to match very closely those produced by anodal polarization, indeed, they could be reversed by cathodal polarization. The pictures, however, were more varied than are those given by polarization due, presumably, to the greater difficulty in obtaining with the solutions a reproducible and regularly graded action along the length of the nerve.

Figure 5 illustrates a case in which the calcium happens to produce successive changes in the picture which in many respects simulate those resulting typically from increasing anodal polarization. The effect of increasing calcium action at the ground lead is illustrated by records *E* to *I*, inclusive. Two successive defections from a diphasic axon potential are shown. They occur in a manner that is more or less typical of the effects induced by increasing anodal polarization at the ground lead, one stage of which, in the same preparation under exactly comparable conditions, is shown in records *A*, *B* and *C*. The anodal increase in amplitude in this case is unusually slight. Treatment with calcium produces here even a slighter increase in amplitude. Furthermore, the increase in the duration of the action potential under treatment with calcium seems to be less than that produced by the anodal polarization. These possibly constitute differences between anodal and calcium actions.

As has been said, defections from the record caused by calcium can, in favorable circumstances, be perfectly reversed by cathodal polarization. An instance of restoration by cathodal polarization of a wave blocked by calcium is illustrated by record *D*.

The records obtained from this axon under the influence of potassium

(*J* to *N*, inclusive) simulate very closely those given by cathodal polarization. The waves that appear on the action potential at a stage just preceding block are lower and their crest times are much shorter than are those produced by anodal polarization; they are shorter even than are those

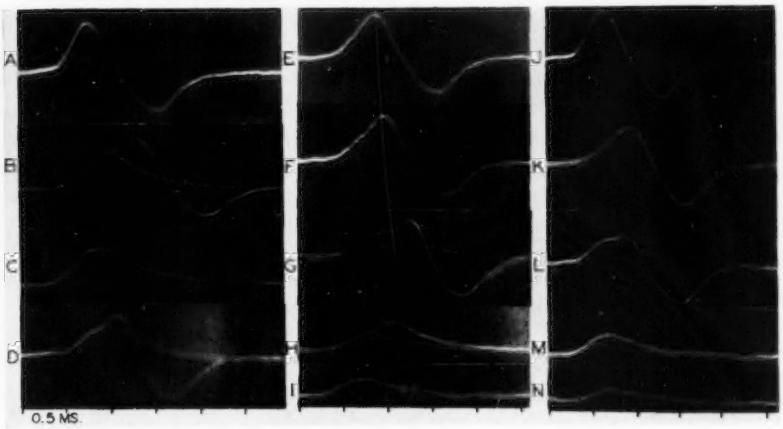

Fig. 5. Comparative effects of anodal polarization and of excesses of calcium and potassium at the ground electrode. All records from the same axon.

A. Diphasic potential, possibly slightly polarized anodally.

B. Solid curve: polarized just short of first block; the ascending limb presents two notches. The dotted curve is *C* superimposed on *B*.

C is the result of blocking the uppermost element in *B*; the record becomes monophasic.

D. Removal of calcium effect by cathodal polarization. After calcium had changed a double-notched, diphasic picture into the single-notched, monophasic record, cathodal polarization converted the latter into the double-notched diphasic record. The two records are reproduced here in superposition.

E. Calcium action beginning.

F. Calcium action, more advanced.

G. Calcium action just short of first block; superimposed on this (dotted) is record, *H*, obtained when the calcium effect was blocking out the element beyond the second notch.

H. Calcium action just short of second block; the first notch of *G* is now later. Superimposed on this (dotted) is the curve *I* obtained when the second block became effective.

I. Action potential after second calcium block.

J. Normal potential.

K. Slight potassium effect; there is some increase in the time to maximum and a notch is beginning to develop.

L. Potassium action just short of first block; superimposed on this (dotted) is record *M*.

M. Record after first potassium block; superimposed on this (dotted) is record *N*.

N. Record after second potassium block.

produced by calcium at a comparable stage. We have not as yet had a satisfactory opportunity to determine whether the potassium effect can be reversed by anodal polarization.

Hypothesis, preliminary formulation. The two sets of pictures we have described, namely, those produced by polarization and those produced by excess of calcium and of potassium can, we believe, be accounted for most readily on the following basis. These agents act on the axis at nodes primarily, but also, though to a lesser degree, on the internodal axis. When anodal polarization, to illustrate by only one of the agents employed, reaches a strength that suffices to block the axis by action on the node most directly exposed to it, its action on the adjacent internodal axis is producing only a moderate effect on the segment response. In other words, block develops at nodes when the internodes, though affected, still are responding. Furthermore, the nodes, we believe, even in normal nerve, constitute physiological discontinuities between internodes which act in effect, perhaps actually, as units. This preliminary statement of our views on the mode of action of the agents we have employed puts us in a position to analyze more in detail the results they have given.

Analysis of a polarization experiment. The diagrams in figure 3 have been drawn to illustrate how the records in that figure can be made to fit the hypothesis outlined above. The supposed composition of the axon potential in the unpolarized axon is diagrammed in *A* of figure 3. The axon is represented by the segmented line. In all of the diagrams the impulse is travelling in the direction of the arrow. In this and in all of the other diagrams the proximal lead rests on segment 3, the grid lead on segment 8. All of the segments develop the same potential in succession but the resulting potential difference that develops between the leads, the recorded potential, the height of which is indicated by the vertical *lines* erected on the segments, depends upon the position of the segments with respect to the leads. For convenience of reference these same lines have been added to all of the other diagrams. For reasons which will be discussed later (see fig. 9), the diagram shows that as activity approaches the ground lead, the recorded potential doubles per segment; the segment that gives the greatest negativity of the proximal lead (indicated upwards, according to convention) usually is the one (in this case the two) closest to the ground lead. The potential recorded from the segments in the interlead stretch will, if it be uniform in diameter, vary along a straight line (Hecht, 1931). Then activity of the segment midway between the leads would leave the leads equally negative and hence will cause no deflection. The records indicated, however, that in this case segment 6 was a bit distal to the equilibrium point. The activity of segment 8 gives to the grid lead its greatest negativity. The effectiveness of segments distal to 8 will diminish with their distance from the lead, but since our pictures tell us little of what is

going on in that locality no further reference will be made to any rôle this region may be playing.

The electrotonic state is indicated diagrammatically by the line *MNO* (see also fig. 9). In the extrapolar regions it changes logarithmically according to a law (indicated by the curve, *MN*) that simulates that used to describe the increase in potential accompanying the approach of the active locus to the lead. In the interpolar region the linear relationship holds in this case as above; but the rate of change (*NO*) with the tripolar arrangement is purely conjectural, since neither the distance between the polarizing electrodes nor the shape of the nerve is known. Contrary to convention, anelectrotonus in the diagrams carries the curve above the baseline, catelectrotonus below it. The height of the segment responses should be increased by anelectrotonus and decreased by catelectrotonus. Depression should be greatest under the peaks of both of the curves. There one would expect the blocks to develop first as polarization increases. In other experiments it has; but in this case the block, with one possible exception (see below), apparently developed, not in node *3–4*, but in node *2–3*. This might signify that the shape of the nerve was such as to condense the current lines here more than elsewhere, or that this node was wider than the *3–4* node. Later a method of measuring the height of the individual responses will be described. In the present instance the height of the several segment responses has been arrived at by inference based on inspection, an admittedly precarious method, and the values thus derived have been sketched into the diagram as the vertical parallelograms.

The point it is desired to make now is that where these parallelograms differ in height from the vertical lines the divergence is in the direction indicated by the position of the polarization curves. Two of our estimations of height require a word of explanation. (a) Record *D* is as high as record *A*, yet in the diagrams the sum of the segment potentials in *D* makes a very much lower action potential than the normal, due to the fact that the polarized potentials of segments *3* and *4* have been drawn very much lower than the normals. This has been done advisedly; the peak of record *D*, we believe, fails to come down under the influence of catelectrotonus, as it does usually, because, though probably the segment potentials actually are lower, the polarization has so shifted the segment responses with respect to each other that the peak is not cut off by the diphasic artifact as soon here as it is in *A*. (b) The other questionable decision has to do with the location of the position of block in record *E*. The noise here is so high and the catelectrotonic depression of amplitude so great that we have been unable to decide whether the block that causes the record to become monophasic develops in node *3–4* or node *2–3*.

Analysis of segment responses. If, as seems likely, the normal axon potential is a composite of unit contributions it would be of interest to

inquire into their time constants and amplitudes, and the manner of their combination to form a normal record.

Segment time can be estimated only when the notches are in evidence. They are brought out best by anodal polarization. In pictures taken at a polarization strength that is sufficient to bring out the notches the notch spacing often is far from uniform. Such is the case, for example, in figure 3, *C*, where wave *3* on the ascending limb of the larger deflection is decidedly shorter temporally than wave *2*. The time between notches, however, is determined in part, at any rate, by local polarization strength; and the fact that in this particular case the block develops between *2* and *3* means that for some reason the *2–3* node is more exposed to the current than is the *3–4* node. Obviously the internotch spaces here are not comparable one with the other.

In order to obtain data that are valid for a comparison of the transit time of an impulse from segment to segment one must, both in the same and in different preparations, measure the time between notches when the anodal polarization is just short of the strength that blocks at the notch that is being measured. We realize in doing this that the position of the notch is to some extent determined by the shapes of the summed parts, and that this cannot always be discounted.

The values thus obtained through measurement of fourteen different segment responses in nine fibers (see table 1) range between 0.33 and 0.72 ms., or 113 per cent. This range, though, includes all experimental errors and all differences in conditions that were uncontrolled or uncontrollable, such, for example, as differences in length of the animals, and temperature, and season; consequently this variation can have little if any significance. Only those segment times are comparable that have been derived from one and the same fiber under the same conditions; and we have available for this purpose only four entirely valid and satisfactory pairs of observations. In these the internotch times in milliseconds were as follows: 0.33 and 0.33, 0.72 and 0.72, 0.35 and 0.37 and 0.58 and 0.43. In another case the times were 0.48 and 0.72, but the latter reading is from a wave that was only 3 mm. high and consequently is very inaccurate. On the basis of such evidence as this it would be precarious to conclude that segment time in a nerve critically polarized anodally is of the same order of magnitude in all segments irrespective of their length. Nevertheless, we believe that the evidence is not opposed to this conclusion.

Shape of the unit potential. The manner of growth and of shifting of the unit contributions to the axon potential as anodal polarization is increased leave little room for doubting that the action potential of unpolarized nerve also is composed of unit contributions from the segments. In normal nerve the successive contributions must be relatively brief and follow each other with very short intervals. This perhaps constitutes the main reason

why contact prints[4] of the unpolarized potential ordinarily give no clear evidence of their presence. There is, however, an additional reason: such prints are not adapted to the reproduction of angles, particularly when there are a number of them following each other closely. Even so, the contact prints of presumably normal axon potentials occasionally do give evidence, inconclusive when taken by itself, of angles in the "foot" of the spike (see fig. 3, *A*), and particularly when, for no obvious reason, the foot is unusually long. But the angles then are much more clearly dis-

TABLE 1*

EXPERIMENT	AMPLITUDE OF SEGMENT RESPONSES	INTERNOTCH TIME	REMARKS
	mm.	*ms.*	
1/16		0.46	
1/19		0.39	Some shifting of base line
1/22	91		The amplitudes here are relative
	50	0.33	
		0.33	
1/24	24	0.58	
1/31		0.59	
3/2		0.72	
4/18	31	0.72	
	18+	0.35	
		0.37	
	3.5		
5/25	30		Polarization changed slightly
	13	0.58	
		0.43	
	9		
5/26	29		Amplitude low; measurement difficult
	10	0.48	
		0.72	
	3		

* When more than one value is given under an experiment each is from a different segment.

cernible in camera pictures. They were seen in camera pictures (two of which are reproduced here in fig. 6) made three years ago when we first began to interest ourselves in axon potentials, but were misinterpreted, having been regarded at that time as indicative of action potentials in fibers that stopped conducting at a point very close to the lead.

Information relative to units in normal nerve cannot be obtained directly,

[4] All of the records reproduced in the figures are contact prints, excepting those of figure 6.

but it is possible to determine their configuration in polarized nerve. Some steps in the procedure are illustrated in figure 7. The normal unpolarized monophasic action potential and base line are seen in *A*; the

Fig. 6. Evidences of segment contributions in unpolarized axons. Photographs. All other records in this paper are contact prints. Time falls off logarithmically from left to right. Temperature 16°C.

A. The time between the two angles is 0.13 ms.; between the second angle and crest, 0.56 ms.

B. The time between the two angles is 1.62 ms.; between the second angle and crest, 0.96 ms. Here the interangle interval is abnormally long, possibly due to damage.

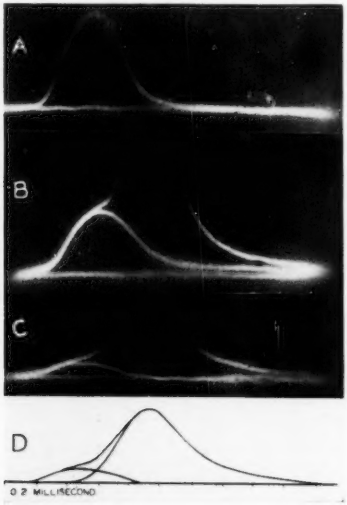

Fig. 7. Three critical stages of anodal polarization at the ground lead.

A. The normal axon potential, monophasic, and base line.

B. The same, polarized to the point where the first defection is taking place, with base line.

C. The lower wave of *B* polarized to the point where the second defection is taking place, with base line.

D. Subtraction of the smaller from the larger deflection in *C*, giving the configuration of a segment response in polarized nerve.

record shows how low the noise level can be when the nerve is unpolarized. The time to maximum is 0.42 ms. In *B* there are three successive sweeps

recorded on one unmoved film,—the high, notched deflection, obtained when anodal polarization at the ground lead was just short of causing the highest element to drop out, the lower curve, when polarization was increased just a trifle so as to block out the highest element, and the base line, made without stimulation of the nerve while maintaining the polarization. Polarization was then increased until the lower deflection of *B* itself broke up as did the original action potential. This gave record *C*. Since any

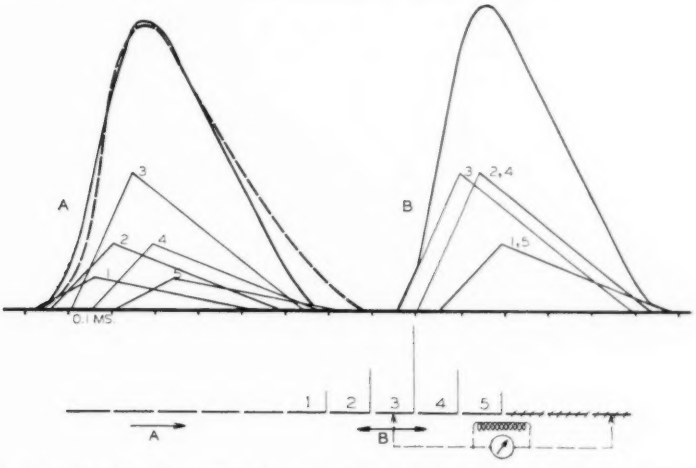

Fig. 8. Showing the assumed composition of the monophasic action potential traced in *A* as the dotted curve. Analysis of this action potential by anodal polarization through the ground lead indicated that the unpolarized action potential conducted from *A* (lower diagram) might be made up of contributions from five segments located with respect to the ground lead as shown in the lower diagram, in which is shown also the probable relative amplitudes of the five contributions. The five units are represented by the correspondingly numbered triangles of like relative heights. They are spaced by the assumed interval (0.05 ms.) between the response of the units in unpolarized nerve. The rising limb of each has a duration of 0.15 ms., the falling limb 0.45 ms., values selected by trial and error so as to yield a sum, the solid line curve, approximating the record, the dotted curve.

In *B* these same triangles are summed (higher curve) in the positions they would occupy if the lead and stimulated point were one and in the position *B* below.

trace of diphasicity the original record might be concealing is removed by the first defection, the difference between the two deflections in *C* must give the shape of a unit response, but, of course, one polarized anodally. The subtraction was made after photographing record *C*, considerably enlarged, on a system of rectangular coördinates. The result is seen in *D*. The unit thus obtained probably is deformed somewhat by the slight difference in anodal polarization at which the two successive records were

made, a difference that was needed in order to insure the obtaining of the two-unit and the one-unit deflections on one film. Other determinations based on deflections made without changing the polarization, yield unit potentials essentially the same in configuration as the one seen here. The rise of the curve obtained by subtraction very definitely is sigmoidal in shape and occupies about 0.5 ms., the decline, a period about 2.5 times as long, the last two-thirds of which is logarithmic. These are typical values.

Segment potential height. The same procedure permits one to determine the relative heights of the unit contributions to the action potential under comparable conditions. The heights of the several elements obtained by subtraction of the records made in each case at the polarization strength needed to cause the defection should give the actual relative heights when multiplied by a factor which varies inversely as the logarithm of the dis-

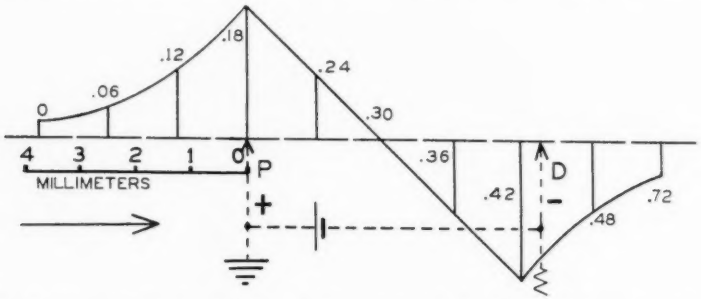

Fig. 9. Diagram illustrating the effectiveness at the leads, P and D, or ground and grid, of potentials originating in the segments (1.25 mm. long) of our typical axon. The recorded heights of the segment potentials is indicated by the vertical lines. The same curve, it is assumed, describes the electrotonic effect of a potential applied to the nerve through P and D.

tance of the contributing part from the lead. The results obtained directly by subtraction, while constant in any one preparation, have varied widely in different preparations. The typical result is illustrated by figure 2. Here the heights, ascertained by subtraction, are to each other approximately as 4:2:1. These are the values that form the basis of the statement made earlier in the paper that the potential falls to half value per segment removed from the lead.

This supplies a clue to the rate at which the potential of the active locus of the axis falls off with the distance along the nerve. From experience we feel reasonably certain that the fibers we have studied conduct at the rate of about 20 m.p.s. and have a diameter of about 12μ. We judge on the basis of Hatai's statistics (1910) that in frogs of the body length of ours the average segment length in such a fiber would be 1.25 mm. But

Bishop, Erlanger and Gasser (1926) and Hecht (1931) have shown that a constant potential applied to a nerve falls to half value in each 2 mm. This discrepancy might be due to the difference in the duration of current flow in the two cases, one being constant and the other momentary; it might also be due to the difference in the sizes of the nerves, for we have gained the impression that the rate of fall of potential is faster in small than in large nerves; but there is also the possibility that segment length is greater in these fibers than we have assumed. Figure 9 has been constructed on the assumption that in our fibers segment length is 1.25 mm. and that the potential of the active locus falls to half value in that distance.

But the deviations from the rule that segment potential height falls to half value per segment have been wide, as may be seen in the following examples: (a) 8.9:5.4:1; (b) 3.1:1.4:1; and (c) 9.7:3.1:1 (see table). Factors to which these variations from the rule might be ascribed are 1, local damage; 2, inequalities in the thickness of the preparation; 3, differences in the thickness of the internodes, and 4, variations in the distances of the successive potential sources (the segments) from the lead. Any differences determined by the first two of the factors mentioned above will be fortuitous and cannot be evaluated. Regarding the third factor, one gains the impression from reading Ide (1931) that variations in the diameter of a fiber are frequent, and that, therefore, the contributions of potential from successive segments might, because of this, differ somewhat in amplitude. Our own measurements (Gasser and Erlanger, 1927) would indicate that this is unimportant.

If the variations in relative height be attributed wholly to the distance factor one can, by referring the above measurements to the logarithmic curve portraying the rate of fall of potential relative to the distance of the segment from the lead (see fig. 9), ascertain, roughly to be sure, the relative lengths of the segments concerned. The 4:2:1 relation signifies, for instance, that the distances separating the potential sources of the first and second, and of the second and third elements are both 1.25 mm. From example *a* (see above) one calculates in this way that the segment intervals (or lengths) are to each other as 15:48, from *b*, 23:12, and from *c*, 32:35. The variations in the lengths of two successive segments of a nerve when measured in this way may therefore occasionally be as great as 300 per cent. This result is not inconsistent with the results obtained by direct measurement (Boycott, 1904; Takahashi, 1908).

It is of interest to compare each of the above ratios with its set of segment times under the same conditions: for *a* they are 0.35 and 0.37 ms., for *b* 0.58 and 0.43 ms. and for *c* 0.48 and 0.72 ms., the last being the questionable measurement. In the case where the amplitude relation was 4:2:1, the segment intervals were 0.33 and 0.33 ms. Taken at its face value, this exhibit signifies that even though the lengths of two segments

of a fiber may differ by 300 per cent the time the impulse spends in these segments may be the same. It definitely indicates that most of the lag caused by anodal polarization is in the nodes, and suggests the thought that this might be the case also in unpolarized nerve. This would in turn point to the conclusion that the longer the segments of a fiber the faster would be the conduction rate in that fiber. This, as a matter of fact, is the case. Furthermore, it is known that the larger the animal of a species the faster is conduction rate and the longer are the segments (Takahashi, 1908). If time is lost mainly in the internodes this would constitute a device for keeping constant the conduction time between center and periphery. It might even mean that segments respond in effect as units. But to settle the matter by this method of approach many more valid observations will be needed than we have been able to accumulate.

The time constants of the normal segment potential. If it be assumed that in normal nerve the time per segment is constant and that the recorded potential falls to half value per node removed from the lead, it becomes possible by the method of trial and error to arrive at an approximation to the time constants of the normal, unpolarized segment response. The method of procedure is illustrated in figure 8, *A*. The dotted curve is the recorded axon potential. An analysis of it by anodal polarization at the ground lead (the grid lead being from the killed end) indicated that the contributing elements were arranged with respect to the active lead somewhat as indicated in the lower diagram. In *A* each of these is represented by a triangle of corresponding height spaced, in their proper order, by 0.05 ms., this being the time the impulse travelling at the rate of 25 m.p.s. remains in a segment 1.25 mm. long. By trial and error it is found that when the rising and falling limbs of these triangles are assigned durations of 0.15 and 0.45 ms., respectively, their sum (the solid curve) approximates reasonably closely the configuration of the actual record. There are, in this case, two discrepancies: 1. The beginning of the summed curve is too high; this possibly is assignable to failure to give to the units summed the sigmoidal rise they probably have. 2. The end of the summed curve falls too rapidly; and this might very well be due to lack of quantitative information relative to the configuration and spacing of the units close to the damaged region. On this basis one reaches the conclusion that the units composing the normal axon potential, as recorded, rise for a period of 0.15 ms. and fall during 0.45 ms. It is realized, though, that the method through which this result has been attained is full of pitfalls.

We may next turn our attention to the matter of *axon insulation*, since this certainly is a factor in the distribution of the effects of polarizing currents, probably also in the manner of spread of current from the active axon to the leads and possibly in other relevant ways, also. The literature is not of material assistance in supplying the information needed here.

Despite the fact that one can readily record nerve potentials and stimulate nerve with applied currents, currents of physiological origin even, myelinated fibers are regarded by some as electrically insulated structures. Thus Göthlin (1910) says that "in so far as conclusions are permitted, the myelin sheath is essentially an insulating structure for small electromotive forces." He attributes the insulation, though, to the cuticular sheaths of the axons rather than to the myelin and he, therefore, believes that the nodes are insulated, though in modified form (what is meant by this is not made clear), as well as the internodes. McClendon (1917), on the other hand, believes that current enters and leaves myelinated fibers only via nodes of Ranvier, and in support of this view shows that with electrodes the same distance apart the irritability of nonmedullated nerve is the same to currents passing crosswise as to currents passing lengthwise; whereas, as is well known, myelinated fibers are scarcely irritable to crosswise currents. However, neither of his arguments in favor of this view is wholly valid. 1. The fact that not all fibers in a nerve are stimulated by a minimal stimulus, which he attributes to variation in the positions of nodes with respect to the electrode, actually is attributable mainly to differences in irritability determined by differences in the sizes of the fibers (Gasser and Erlanger, 1927). 2. The lower threshold of myelinated fibers to lengthwise than to crosswise currents he attributes to differences in the number of nodes affected by the current. But in Rushton's experiments (1927), for example, the same difference in irritability was demonstrable when, with changing direction of current through the nerve, the length of nerve exposed to the current was kept constant. In our opinion a continuous longitudinal resistance that is higher in myelinated than in unmyelinated fibers would account satisfactorily for McClendon's results, particularly if it be true (Rushton, 1927) that "excitation is brought about by the current leaving the nerve through the cylindrical sheath." The question, then, of the relative resistivity of nodal and internodal sheaths still remains unanswered.

Our own evidence indicates that fibers in their natural position and state are sufficiently well insulated to be effectively insulated from each other, and that nodes are less well insulated than are internodes. Thus, relative to insulation of fibers from each other, we have found (Blair and Erlanger, 1932) that the irritability of the fibers of a nerve is not measurably raised by any eddy currents that may be originating in neighboring active fibers. And yet, when our observations on the action of polarization and of salts are considered in relation to each other, one cannot avoid the conclusion, to which reference has been made in passing, that these agents act primarily through nodes and block there, but also have an effect on the internodes. That the chemicals act primarily on nodes seems unequivocal. It has been established (Gasser and Erlanger, 1929) that thin fibers re-

spond to the action of chemicals more rapidly than thick fibers. And de Renyi (1929) has shown that the node itself has about half the diameter of the internodal axis. Furthermore, since the sheaths covering the nodes, being free of myelin, are thinner than the internodal sheaths, diffusion of chemicals to the axis must be considerably freer in nodes than in internodes. But the pictures produced by anodal polarization resemble so closely those produced by calcium action that there can be no escape from the conclusion that both act on the same locality. This is indicated also (as has been seen), but not proved, by the fact that the salt action is reversible through appropriate electrical treatment. Furthermore, if the blocking action of these agents were through effects exerted on the axis anywhere the pictures could not be as much alike as they are in different nerves, nor as constant as they are in one and the same nerve, unless perchance, segments could be thrown out of action as units. It is fortunate that this evidence indicating that currents block at nodes is available, since there is on record indirect evidence which otherwise would incline one to conclude that currents act primarily on internodes. This is the fact that ordinarily the larger the fiber the more susceptible it is to the action of a constant (or any other) current; the larger the fiber the lower is its threshold to electrical stimulation and the greater its susceptibility to electrotonus.

But though the constant current blocks at nodes it at the same time seems to be exerting an effect upon the internodal axis, and presumably through its sheath. Thus the height of the recorded wave increases continuously during increasing anodal polarization up to block. If the potential we are recording originated primarily in the region whose activity is blocked one would expect the potential from it to fall off shortly (at least) before block. Since the reverse actually is the case one is forced to conclude that while the current is blocking by action in a node it is exerting a less than maximal effect on the internodal axis. The myelin sheath, in other words, is not impermeable to applied potentials.

These observations have a very direct bearing on the source of the waves produced by the agents we have used. Thus it is conceivable that they are produced by potential escaping from uninsulated nodes as the active locus issuing from the insulated internodal axis passes by them. But it can, we believe, be shown that this is highly improbable. According to de Renyi, the nodal axis is constricted to about half the diameter of the internodal axis, and his pictures show that the gap in the myelin is less, probably considerably less, than 6μ in a 12μ fiber. The length of the segment he does not give. If it be put at 1 mm., the area of the internodal axis would exceed that of a node considerably more than 167 times. Therefore, to reduce to equality the number of current lines escaping to the leads from these two parts of the axis the difference in the resistance of

their sheaths would have to be at least as great as this figure. The presence of the Schmitt-Lantermann incisures, the evidence we have just presented indicating that applied potentials act on both the nodal and the internodal regions, though to the sure, less on the latter, and the opinion of Göthlin that the sheath resistance is not primarily due to the myelin, all combine to indicate that the difference in resistivity of these two parts is not sufficiently great to overcome the difference in the area factor.

What is the significance of these considerations relative to past efforts to ascertain *the shape of the axon action potential*? Only those investigations that have employed the cathode ray oscillograph will be considered here because that instrument ". . . . is capable of giving more accurate records of nerve action potentials than have been obtained with any other instrument . . . ." (Matthews, 1928). For the purpose of determining the shape of the axon spike a number of procedures have been employed, none of which, it can be shown, partly in confirmation of previous surmises (Bishop and Erlanger, 1926; Bishop, 1927), could possibly have yielded entirely valid pictures. At a stage when adequate records could not be obtained from single axons the sum of the potentials of the many fibers responding to a brief submaximal shock applied at (Erlanger and Gasser, 1924; Bishop, 1927) the proximal lead was supposed to give the time constants of the axon potential; for it was then believed that all of the axon potentials thus summed are alike temporally, and that to obtain them all perfectly superimposed it was necessary only to obviate conduction and so the temporal dispersion that results from differences in conduction rate. We now know, however, (a) that fibers do not all respond to a shock with the same latency (Blair and Erlanger, 1933) and that their spikes, therefore, cannot all be exactly superimposed even when conduction is eliminated; (b) that as the active locus moves in *either* direction from the stimulated site, in this case the ground lead, the potential from it will continue to affect the lead, though decrementally, as indicated in *B* of figure 8; and it will be shown (c) that such leadbacks are longer temporally the slower the conduction in a fiber, a fact that prevents exact superposition of the potentials of a multifiber response. Finally (d) one never can be sure, partly because of the spontaneous play in the irritability of a fiber, that one is always stimulating the point on the fiber directly over the stimulating electrode and consequently directly over the lead (Blair and Erlanger, 1933). For these and for other reasons, it is clear (a) that the time constants and configuration of the potential developed locally by activity in an axon cannot be ascertained directly by methods that involve leading responses, either multifiber or single fiber, from the nerve; and (b) that the durations obtained by such methods are bound to be very much longer than are those of our unit response. The time values of the unit or segment response represent, we believe, the nearest approach yet attained to

those associated with local activity in nerve; and it is not unlikely that they still are much too long.

Does stimulation occur through nodes? If electrical currents act primarily on the nodes stimulation by applied current should take place there. One would then expect the spread of current that occurs upon increasing stimulation strength to cause the shock-spike (conduction) time to diminish in steps equal to the segment time. We have attempted to ascertain whether this occurs, but without success. If there is a step-like progression of the locus stimulated the steps would be of the order of 0.06 ms. But latency of the response to a shock at threshold is about 0.2 to 0.3 ms., and at threshold varies irregularly through a range at least as wide as 0.1 ms. It is obvious that it would be impossible to recognize steps of 0.06 ms. through a vacillation of 0.1 ms. or more, and it is this, presumably, that accounts for our failure.

Discussion. We have now considered from the standpoint of what seems to be the most plausible hypothesis the alterations effected in the configuration of the axon potential by the agents we have employed and have discussed their significance relative to normal conduction. Attention may be directed now to certain other possible ways that have occurred to us of accounting for the results.

1. If the myelin sheath is the complete insulator it is believed by some to be, and if the nodes are uninsulated, it would be necessary to consider the possibility that the segmental increments of the axon potential are produced by successive leaks to the leads of potential from the active locus as it passes nodes. One observation that seems to invalidate this possibility has been referred to specifically. It is invalidated also by most of the arguments used above in support of our first hypothesis. It will be unnecessary to direct attention to these again.

2. The pictures could be accounted for if progression were saltatory and by a process such as Lillie (1925) has described as occurring in the iron wire model in which the immersed wire is enclosed in a segmented glass tube. Here, due to reactivation by eddy currents flowing around the segments, activity progresses in jumps from node to node and consequently is more rapid than in the simple model. Lillie has suggested that conduction might proceed in this fashion in myelinated fibers. On the basis of certain considerations, however, we are inclined to think unfavorably of this as a mechanism for propagation in nerve. Propagation of this kind would have to be effected through restimulation by eddy currents flowing from node to node *around* the segments. In other words, impulse propagation in a fiber would be determined by a process that operates through structures that are foreign to the fiber. It seems much more reasonable to suppose that the nerve fiber as a conductor is a self-contained mechanism; that it contains within itself everything that is necessary for

the performance of its own proper function. There are, however, other, perhaps more cogent, reasons for regarding this as an unlikely mechanism. Thus the myelin of fibers of the posterior columns of the cord is unsegmented; yet those fibers can conduct *as rapidly* as peripheral segmented fibers (Gasser and Graham, 1933). And there is evidence, already referred to, indicating that the eddy currents that are supposed to develop in nerve are without physiological significance.

3. The possibility also has occurred to us that by our procedures we may be disclosing saltatory progression of another, still ill-defined, type. Histologically, segments might well be relatively insulated boxes. This they would be if the nodal beads described by de Renyi had the electrical resistivity of the myelin sheath, the edges of which, according to him, are closely applied to the beads. Then, if propagation were by a combination of chemical and electrical processes, the potential pattern of the chemical process initiated anywhere in such a box would, since electrical leak is insignificant, be imparted to the whole of the box from any locus within as rapidly as capacity could be satisfied; and if the attainment of maximum potential acted to limit the reaction, it is obvious that a minimal liberation of energy would suffice for action. The propagation of the chemical process along the length of the segment would not be necessary for the progress of the nerve impulse. Propagation from segment to segment then would have to be effected by some device for transferring energy across the junction which, in the present state of the problem, it would be futile to discuss.

From the standpoint of teleology the development in the fastest of the fibers, as they become myelinated, of intercalated, slowly-conducting, disks would be incomprehensible if their introduction were not accompanied by other changes that more than compensated the reduction in speed. The conversion of steady into saltatory progression by either of the two mechanisms mentioned above would do just that.

The fact that in nodal block produced by anodal polarization the interval between the crest of the segment response to remain and the start of the segment response about to be blocked often (see fig. 4) is about equal to, and never exceeds, the value that can be accounted for by the latency of response, strongly suggests that we are dealing with a saltatory process in which the limiting interval is the time to maximum of the unit segment response plus the latency of the next segment. It may be of interest in this connection to call attention to the fact that the normal segment time in our fibers, namely, 0.06 ms., is the response latency of these fibers to a shock; if propagation is by restimulation this interval leaves no time for conduction through the internodal region.

SUMMARY

By graded treatment of nerve with anodal or cathodal polarization or with Ringer's solution containing an excess of calcium or of potassium it is possible to develop on the conducted axon potential a series of waves delimited by notches, and to block out parts of the action potential by units consisting of these waves. By following the effects of anodal polarization as a type it can be shown that these agents bring into view by one process or another a notching that is normally present.

The waves can be variously accounted for, but are believed to be the responses of segments, and the intervals between them are believed to be determined by a lag in transmission across nodes. It is assumed that this lag is the result of a physiological discontinuity determined by local constriction and possibly by differences in the physiological properties of nodal and internodal axis that are indicated by differences in the consistency of these parts. The possibility is considered that segments act as units.

As determined by trial and error the normal segment potentials of fast fibers are believed to have rising and falling times of not over 0.15 and 0.45 ms., respectively. The rising and falling times of the conducted spike of any axon will be the rising and falling times of its segment response plus the time for conduction of the impulse the distance of the appreciable action potential spread, which, on the basis of the present experiments, is put at about 4 mm.; the potential falls to half value in 1.25 mm. in these small nerves. There is no way yet apparent of determining segment response time directly in normal nerve.

Evidence indicates that nodes are less well insulated than internodes, both electrically and chemically. It is for this reason, presumably, that anodal polarization, for example, blocks at nodes at a strength which is enhancing and prolonging the internodal response.

Calcium block can be removed by cathodal polarization.

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MIDDLE EAR PRESSURE AND AUDITORY ACUITY[1]

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A series of sixty experiments has been carried out to determine the effect of changes in middle ear pressure on the transmission of sound stimuli. As in the previous observations on the function of the various structures of the cat's ear reported from this laboratory during the past three years (1, 2, 3) the Wever and Bray phenomenon has been used as a means of determining the effect of the different experimental procedures. The only significant observations on increased and decreased middle ear pressure have been made upon human beings (Fowler, 4; Pohlman and Kranz, 5, Békésy, 6). These investigators have in each case used some method of increasing pressure in the external auditory canal while testing the hearing. No direct measurements of middle ear pressures have been possible under their experimental conditions. Obviously no equalization could take place through the eustachian tube under these circumstances. It would seem probable, therefore, that whatever diminution in hearing resulted was due to fixation of the drum rather than to any real change in middle ear pressure. The results of experiments on cats already reported (2) in which fixation of the drum and ossicles was obtained by tension applied to either the tensor tympani or stapedius tendon correspond very closely to the results of these observations on pressure changes in the external canal of human beings. Many clinical reports, assuming negative or positive middle ear pressure to exist and detailing treatment for its correction, have been made. This latter phase of the subject will not be discussed here aside from mentioning the well-known fact that sudden changes in atmospheric pressure do cause a temporary impairment of hearing which is relieved immediately as external and middle ear pressures are equalized.

No material is available which indicates in any way the amount of pressure changes in the middle ear resulting from occlusion of the eustachian tube.

EXPERIMENTAL METHOD. The experiment is set up in the manner already described in detail, ether anesthesia being used. An electrode is

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placed in each auditory nerve and the usual series of control tests made before the apparatus for producing and measuring pressure changes is attached. Two methods have been used to measure middle ear pressure. In the first a manometer needle was inserted in the mastoid bulla and after the eustachian tube had been blocked pressures were changed and transmission tests made. The second method consisted of the following procedure. After exposure of the bulla a small opening, of the same diameter as the recording manometer needle, was drilled through the bony wall. This needle, of a length designed to prevent its impinging against the inner wall of the bulla, was inserted through the hole in the bulla and made secure with bone wax. The animal's mouth was then opened widely, the tongue retracted and a midline incision made through the soft palate exposing the orifices of the two eustachian tubes. After bleeding was controlled and all mucus carefully removed from the naso-pharynx a needle connected with the pressure manometer was inserted into the eustachian tube on the same side. As the eustachian tube in the cat enters the middle ear at an angle and the opening is small it was found necessary to use a specially designed curved needle which could be introduced along the medial wall without risk of injury to any of the middle ear structures. Difficulty was experienced at first in attempting to produce negative pressure due to the fact that the needle became blocked with mucus. This difficulty was met, however, by inserting the needle under slight positive pressure, thus forcing ahead of the needle opening any mucus that might be present in the tube itself. When properly placed the needle end lay free in the anterior part of the middle ear cavity. Following insertion of the two needles, control tests were repeated with normal middle ear pressure and in no instance was transmission affected unless injury had been done to some middle ear structure in which case no further tests were carried out on the damaged side. Positive and negative pressures were obtained by means of an electrically driven pump, the degree of pressure change being governed by the speed of the motor. The maximum pressures available with this apparatus are plus or minus 30 mm. of mercury. Greater changes if desired may be obtained by connecting the pressure manometer directly with a compressed air line or water suction apparatus. The test of a properly functioning manometer system involved a reading in the recording manometer corresponding to that in the pressure manometer and immediate return to normal on both sides of the system following return to normal pressure. If this test failed to be met at any point in the experiment, blockage of the eustachian needle was usually the cause.

As a basis of comparison the various frequencies are impressed on the cat's ear at an intensity ranging from 75 to 90 decibels above average human threshold. This intensity is referred to as "normal" and is 15 decibels below the maximum which can be obtained from the continuous pitch

range auditometer for 180, 250, 500 and 4000 d.v. while for 1000 and 2000 d.v., 25 decibels below maximum is used. In the routine experiments transmission tests were made for all frequencies at four different intensities, the lowest or initial intensity being 30 decibels below normal. The intensity was then increased 10 decibels for each succeeding test through normal. Large changes in middle ear pressure from 30 to 50 mm. of mercury were first used and it was found that such pressures, whether positive or negative, impaired transmission to approximately the same extent. In general the greater the pressure change the more marked was the impairment. The relative amount of impairment, however, varied in different animals. The frequencies 180, 250, 500 and 8000 d.v. were affected more than 1000, 2000 and 4000 d.v. and in some cases with small negative pressure changes, 4000 d.v. even showed improvement over the control values. This was not a constant finding, however. At initial intensity the effect, impairment of all tones, was relatively more marked than at normal intensity. After every change in middle ear pressure control tests were made with middle ear pressure normal and in practically every instance transmission returned to its original control level. Data from a typical protocol illustrates the order in which these tests were carried out.

Protocol 1. Left ear

PROCEDURE	ATTENUATION	180	250	500	1000	2000	4000	8000
Control	−30	30	29	26	36	36	42	
	−20	22	21	23	33	34	40	53
	−10	20	24	20	30	33	44	45
	Normal	20	20	20	33	35	49	47
Manometers in place, pressure increased 20 mm. Hg	−30			41	50			
	−20			34	49		60	
	−10	46	43	34	47	57	50	
	Normal	34	34	33	40	46	44	
Pressure zero	−30	30	30	30	37	35	43	*
	Normal	19	20	23	30	37	50	49
Pressure decreased 30 mm. Hg	−30			44			44	
	−20		40	39		52	39	
	−10	37	37	40	51	40	37	49
	Normal	32	30	32	40	35	38	45
Pressure zero	−30	34	30	30	39	37	39	60
	Normal	19	20	20	31	35	43	44

* Too faint to balance.

A group of experiments was done to determine the minimum amount of pressure change necessary to produce a change in transmission. As the lower intensities were more sensitive to changes in middle ear pressure only the initial intensity test (30 decibels below normal) was used. Middle ear pressure was increased from normal 5 mm. of mercury at a time up to 20 mm. Slight impairment occurred with 5 mm. of mercury while with

each 5 mm. increase the transmission became correspondingly poorer. Similar results were obtained when the same procedure was carried out with decreased pressure.

With changes in pressure of 30 mm. of mercury, whether positive or negative, practically no tones were transmitted at initial intensity while at normal intensity in most cases only 250 and 8000 d.v. were lost. Although the degree of impairment varied in different animals, nevertheless in every instance the tendency towards progressive impairment with each succeeding pressure change was clearly manifest.

Fatigue. In order to analyse this response to middle ear pressure changes more fully the method of measurement described (7) was used in a number of experiments. In this procedure the ear is exposed to a series of stimuli of gradually increasing intensity for all frequencies. After the maximum intensity available from the sound source is reached all the tests are repeated in reverse order. The decrease in response following stimulation with maximal intensities represents true fatigue of the ear. Each series of tests represents thirteen complete transmission measurements. All normal ears show this fatigue effect when subjected to such sound stimuli.

When the middle ear pressure was increased 30 mm. of mercury the same procedure was carried out. Though the general level of transmission was markedly reduced by this pressure change no fatigue developed. The pressure was then released and the ear again exposed to the fatiguing tones with normal middle ear pressure; transmission for all intensities up to the normal level returned to control values but following full intensity the usual fatigue effect developed. Similar experiments were carried out with pressure decreased approximately the same amount. With this negative middle ear pressure the ear again failed to fatigue but with restoration of the pressure to normal, fatigue developed as usual.

This type of test was carried out in a considerable number of experiments using smaller changes in pressure and it was found that the amount of fatigue varied inversely with the amount of middle ear pressure change. The fatigue effect, when present, persisted even after middle ear pressures were restored to normal. In the majority of experiments it was necessary to use changes in pressure either positive or negative of at least 30 mm. of mercury to eliminate fatigue completely.

Relation to inner ear pressure. As previously reported (8) decreased intralabyrinthine pressure results in an impairment of transmission in the experimental ear, while an increase in pressure has little, if any, effect. If intralabyrinthine pressure were changed sufficiently by changes in middle ear pressure to be a factor in the reduced transmission the effect might be compensated for by changing the inner ear pressure in the opposite direction. To determine whether or not this might be a factor the following procedures were carried out. After the manometers were in position control tests with normal, increased and decreased middle ear pressures were

made. In some animals intralabyrinthine pressure was then decreased by an intravenous injection of 10 cc. 30 per cent sodium chloride solution, in others intralabyrinthine pressure was increased by intravenous injections of 50 cc. distilled water. Following the injections tests were made with normal, increased and decreased middle ear pressures. Protocols of two typical experiments follow.

Protocol 2. June 22, 1934: Attempt to compensate for middle ear pressure changes by decreasing intralabyrinthine pressure. Intravenous injection 9 cc. 30 per cent sodium chloride.

Anesthesia: Intratracheal ether. Started 9:30 a.m.; animal sacrificed 3:00 p.m.

Procedure: Animal prepared for test in usual manner, electrodes being placed on both auditory nerves. Ground electrode placed in back of neck. Both bullae exposed but not opened. After control tests were made the manometers were put in place in the left ear, one needle being placed in the eustachian tube and the other inserted through a small hole in the bulla. Pressure tests were made and the animal given an intravenous injection of 9 cc. 30 per cent sodium chloride.

Results of left ear

TIME	PROCEDURE	ATTENUATION	180	250	500	1000	2000	4000	8000
10:10	Control	−30	39	30	27	33	38	40	59
		−20	30	24	23	30	35	38	57
		−10	24	24	24	33	37	40	50
		Normal	23	22	20	42	50	49	47
10:45	Manometers in place, pressure	−30						*	
	increased 32 mm. Hg	−20		57	40	50		47	
		−10		44	40	49	60	45	
		Normal		42	37	46	47	39	
10:50	Control, pressure zero	−30	39	33	28	36	37	37	59
		Normal	22	23	24	36	36	46	49
10:53	Pressure decreased 32 mm. Hg	−30							
		−20		50	57			*	
		−10		50	54			56	60
		Normal		50	48	48		40	47
11:00	Control, pressure zero	−30	39	33	30	30	42	41	50
		Normal	24	23	23	39	40	43	49
11:17	Injection started								
11:30	Injection ended								
1:45	Pressure zero	−30							
		Normal	40	43	40	46			
1:50	Pressure increased 32 mm. Hg	−30							
		Normal							
1:52	Pressure zero	−30							
		Normal	41	43	38				
1:55	Pressure decreased 32 mm. Hg	−30							
		Normal							
1:57	Pressure zero	−30							
		Normal	40	45	41	*			

* Too faint to balance.

Note: As the maximum change in intralabrinthine pressure does not occur until some time after the injection only the results showing the maximum change in transmission are given above.

Protocol 3. June 15, 1934: Attempt to compensate for middle ear pressure changes by increasing intralabyrinthine pressure. Intravenous injection 50 cc. distilled water.

Anesthesia: Intratracheal ether. Started 9:30 a.m.; animal sacrificed 1:00 p.m.

Procedure: Animal prepared for test in usual manner, electrodes being placed on both auditory nerves. Ground electrode placed in back of neck. Both bullae exposed but not opened. After control tests were made the manometers were put in place in the right ear, one needle being placed in the eustachian tube and the other inserted through a small hole in the bulla. Pressure tests were made and the animal given an intravenous injection of 50 cc. distilled water.

Results of right ear

TIME	PROCEDURE	ATTENUATION	180	250	500	1000	2000	4000	8000
10:25	Control	−30			42		40	*	
		−20		43	40	48	34	50	
		−10	38	37	34	33	34	43	56
		Normal	34	37	20	27	35	43	60
11:12	Manometers in place, pressure	−30						*	
	decreased 30 mm. Hg, fell to	−20			47	53	43	61	
	15 mm. Hg	−10		50	48	53	42	56	
		Normal	36	39	30	35	39	47	61
	Eustachian tube needle removed,	−30		50	45	39	43	*	
	cleaned out and replaced, control, pressure zero	Normal	25	27	27	36	36	46	50
11:25	Pressure increased 30 mm. Hg	−30							
		−20							
		−10							
		Normal						59	
	Control, pressure zero	−30			43	40	54		
		Normal	26	30	31	35	37	43	53
11:45	Injection started								
12:10	Injection ended								
12:50	Pressure zero	Normal	28	30	29	27	34	46	62
	Pressure increased 30 mm. Hg	Normal						60	
	Pressure zero	Normal	28	27	30	31	34	45	*
	Pressure decreased 30 mm. Hg	Normal		47	45	54	40	47	65
	Pressure zero	Normal	27	27	29	31	37	45	56

* Too faint to balance.

No evidence whatever of a compensatory effect could be obtained in either case. Regardless of the middle ear pressure the injection of the sodium chloride solution caused a falling off in the intensity transmitted and with the distilled water little effect could be observed. It would seem fairly evident, therefore, that the changes produced by increase or decrease in either middle or inner ear pressures are independent of each other.

Discussion. In the absence of any previous observations of the actual effect of measured changes in middle ear pressure on the functional ability of the ear the facts presented above warrant discussion. Pressures applied

to the drum through the external canal must serve only to fix the drum and in consequence the ossicular chain, an effect corresponding in every way to tension upon the tendons of the intrinsic muscles. In this latter type of experiment there could be no change in middle ear pressure due to the fact that the bulla must necessarily be opened. The same explanation may of course be used in regard to the present experiments which however are based on measured middle ear pressure changes.

A point of considerable interest develops in the minimum amount of pressure required to produce a significant effect upon the ear, namely, 5 mm. of mercury. The pressure changes used in this series of experiments correspond to differences in atmospheric pressure ranging from 1180 feet below to 1140 feet above sea level, the minimum pressure representing approximately a 300 foot variation. Sudden pressure changes of course have a definite and well recognized effect upon auditory acuity. This acute change may result in a temporary impairment of hearing or actual damage to the inner ear depending upon the degree of pressure exerted. In none of the experiments reported was there a permanent effect from the pressure unless it was of sufficient extent to actually rupture the drum. This seldom occurred unless pressures of 60 to 70 mm. of mercury were used. It is difficult to believe therefore that any pressure change could develop in the middle ear as a result of occlusion of the eustachian tube which could change normal pressure relations sufficiently to impair hearing. In the authors' experience there is certainly no other closed body cavity which by absorption or production of air can produce changes of pressure equivalent to 5 mm. of mercury above or below that usually present. It would seem more likely that actual damage of some sort must be done to the conducting mechanism of the ear as a result of such occlusion.

Finally the analogous effect of middle ear pressure changes and muscle tension upon the development of fatigue again indicates a conduction effect. In the same way the inner ear pressure change fails to modify the impaired transmission.

CONCLUSIONS

1. Either increased or decreased middle ear pressures, determined by direct measurement, impair the functional ability of the ear to approximately the same extent. This degree of impairment is directly proportional to the amount of pressure exerted.

2. Plus or minus 5 mm. of mercury was found to be the minimum pressure change which would produce impairment in transmission. Low and high frequencies are affected more than those in the middle range.

3. Variations in middle ear pressures have no apparent effect on inner ear pressure nor is this effect modified by actually changing inner ear pressure.

4. The effect of middle ear pressure change seems definitely directed against the conduction mechanism of the ear.

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VOLUNTARILY INDUCED INCREASES IN THE RATES OF CERTAIN "INVOLUNTARY" PHYSIOLOGICAL PROCESSES OF A HUMAN SUBJECT

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In a series of measurements of basal metabolism over long periods of time and with a variety of subjects, unexplainable variations in the range of metabolism, either on the same day or on different days, are occasionally found. This was brought out markedly in a recent study of the basal metabolism of schizophrenic patients (6) in which the ranges varied from 2 to 72 per cent in the extremes on 214 subjects. It was the belief of one of us (R. G. H.) that he could alter voluntarily the rate of oxygen consumption without an observer's being aware that any effort was being made to change the physiological processes. This belief was based in part on antecedent knowledge that his pulse rate and systolic blood pressure were in considerable measure amenable to "voluntary" control.

Arrangements were instituted whereby measurements of his respiratory exchange were made preceding, during and following periods in which there was voluntary effort on the part of the subject to increase the metabolism. In a preliminary experiment in which the Benedict closed circuit respiration apparatus with helmet was used, the oxygen absorption in three normal periods ranged from 198 to 206 cc. per minute and in the two periods in which a voluntary effort was made, the oxygen absorption averaged 314 and 240 cc. per minute for periods of 9 and 13 minutes, respectively. The respiration, however, was so irregular in the periods of voluntary increase that it was difficult to judge of the accuracy of the measurement since this depends upon the uniformity of the volume of the lungs at the end of expiration. Consequently arrangements were made to continue the investigation with a different procedure.

The respiratory exchange was measured by the open circuit principle using a helmet (1), two dry gas meters, the Fox bag method (4) of aliquot sampling and the Carpenter gas analysis apparatus (5). Attempts were made with only partial success to obtain a continuous record of the heart rate by the Boas cardiotachometer (3). The blood pressure was obtained frequently by means of a Tycos instrument and the pulse rate by count

from the wrist. In the early experiments the rectal temperatures were obtained by means of a thermocouple (2).

As far as we are aware there is no study recorded in the literature in which as many factors were measured at the same time as with the subject of this study. Lev and Hamburger (7) reported a change in basal me-

TABLE 1

Results of experiments on voluntarily increased physiological processes

(Values per minute)

DATE	DURATION OF PERIOD	CONDITION	O_2	R.Q.	PULSE	RESPIRATION RATE	BLOOD PRESSURE	
							Syst.	Diast.
1933	*mins.*		*cc.*				*mm.*	*mm.*
July 18	10	Normal	213	0.78	58	6.2	121	76
	10	Normal	209	0.82	57	7.5	112	75
	10	Increased	*249*	*0.87*	*68*	*8.6*	*140*	*95*
	10	Normal	222	0.83	60	6.6	117	78
	10	Normal	214	0.81	59	6.6	112	75
July 25	10	Normal	211	0.81	57	9.7	101	75
	9	Normal	210	0.85	56	9.4	102	77
	10	Increased	*266*	*0.89*	*69*	*11.9*	*130*	*95*
	10	Normal	209	0.80	57	9.3	105	74
	10	Normal	210	0.74	56	9.5	107	76
Aug. 1	10	Normal	209	0.81	53	11.2	103	79
	10	Normal	218	0.80	55	9.6	106	79
	10	Increased	*283*	*0.96*	*68*	*14.2*	*134*	*100*
	10	Normal	221	0.74	58	11.6	111	73
	10	Normal	224	0.73	56	11.3	104	76
	10	Increased	*307**	*0.93*		*15.4*		
Aug. 8	10	Normal	209	0.83	54	12.6	102	77
	10	Normal	207	0.84	54	12.7	97	76
	5	Increased	*242*	*0.84*	*66*	*11.8*	*109*	*81*
	10	Normal	223	0.84	55	10.6	97	74
	5	Increased	*234*	*0.83*	*66*	*10.8*	*113*	*80*
	10	Normal	220	0.86	55	12.5	97	73
	10	Normal	211	0.82	54	12.8	98	75

* Voluntary contraction of muscles permitted.

tabolism from −6 per cent to +5.1 per cent with a patient who could produce tachycardia by turning over on his right side.

The subject of this investigation was 53 years old, 74.6 kilos nude weight, and 179 cm. in height. He came to the Laboratory without breakfast and took off all his clothing with the exception of a pair of running trunks. He lay down on a couch and was then covered with a blanket, the cuff of

the blood pressure apparatus was attached and the rectal thermo-element inserted. After a preliminary rest period the helmet was placed on the subject, ventilation started and a 15-minute preliminary ventilation carried out without any measurement. The first period was then begun, and the periods were carried out in succession according to a schedule previously arranged with the subject. He was told at the beginning of each period the designating number of the period and, during the period in which he attempted the voluntary increase in metabolism, he was notified as soon as the time was completed for the period. The results of four experiments of this character are shown in table 1 in which are given the duration of the period, the condition of the subject (normal or voluntary increase), and the values of the observations on gaseous exchange, pulse and respiration rates, and systolic and diastolic blood pressures. The subject was under the initial impression that changes in the heart rate and the systolic pressure could be voluntarily divorced from changes in skeletal muscle tonus. That is to say, he knew that such changes could be produced while maintaining a conscious perception that the muscles were flaccid—a perception verifiable by palpation.

On July 18, the rectal temperatures were measured and for the successive periods were as follows: 36.4, 36.4, 36.4, 36.5° and 36.5° C. On July 25 the rectal temperatures varied slightly more, rising from 36.4 in the first period to 36.7 in the last period. It thus appeared that the increases in the oxygen consumption rate were not accompanied by significant rises in body temperature. During the last period on August 1 the blood pressure apparatus and the electrodes of the cardiotachometer were removed, and an effort was made by the subject to produce as large a change as possible. It was his purpose not to inhibit muscle tonus but to maintain a deceptively unchanged posture so that the tonus would not be readily perceptible to the observers. He felt that the periodic taking of the blood pressure distracted him from his voluntary effort and he believed that he could produce a somewhat greater rise were no apparatus attached to him except the helmet. It will be seen that there is a slightly greater rise in the oxygen absorption in the last period—in which the subject made no attempt to inhibit skeletal muscle tonus—than in the third period in which the attempt was made to keep the voluntary muscles flaccid. The difference, however, between these two periods is not so large as the difference between the initial control period and the experimental (third) period. The increment of oxygen consumption due to the "permitted" muscle tension was thus only 307 minus 283 or 24 cc. per minute. On August 8, the periods of voluntarily increased metabolism were shortened with the idea of accentuating the maximum rise. However, the rise in the two five-minute periods of voluntarily increased metabolism was not so great as in the other three experiments in which the periods of voluntarily increased

metabolism were longer. Before he had been informed as to the results the subject volunteered the information that he was conscious of having been less successful than in previous experiments.

In table 2 are shown the percentage rises in the various factors observed when the first two periods on each day are used as a basis of the calculation of the percentage increases in the factors. There is a fairly good agreement among the percentage rises in oxygen consumption, pulse, systolic and diastolic pressure and respiration rate in each of the first three experiments. In the fourth experiment there is a good agreement between the percentage increases in oxygen consumption and pulse rate, but not so good with regard to the blood pressure. Since considerable energy expenditure was required to augment the heart rate coincidentally with augmented blood pressure and since presumably much less expenditure was required for the

TABLE 2

Percentage increases in the physiological processes as the result of voluntarily induced changes

DATE	O_2 PER MINUTE	R.Q.	PULSE	RESPIRATION RATE	BLOOD PRESSURE			
					Syst.		Diast.	
1933					*mm.*	%	*mm.*	%
July 18	18	9	17	25	23	20	19	25
July 25	26	7	21	24	28	27	19	25
Aug. 1	32	20	26	37	29	28	21	27
	43*	15		48				
Aug. 8	16	1	22	−7	9	9	4	5
	13	0	22	−15	13	13	3	4

* Voluntary contraction of muscles permitted.

changes in peripheral vascular resistance by which the increased pressure was rendered possible this lower correlation was to have been expected.

That the augmented oxygen absorption was not in any significant degree dependent upon increased respiratory effort is suggested by the fact that, in the fourth experiment, the respiratory rate was even decreased. The subject, incidentally, has the subjective impression that the respiration rate can be to a considerable degree controlled independently of the other processes studied. The relative rise in the respiratory quotient in all of the experiments was not so great for the most part as in the other factors. An increase in the quotient is not to be expected unless one assumes that the augmented metabolism is either completely or partially due to the increased combustion of carbohydrates. There is no reason to suppose that such a small increase in metabolism relatively as compared with that

which might take place during muscular work would occur solely at the expense of carbohydrates. The higher respiratory quotients during the periods of voluntary increase are probably ascribable to the incidental over-ventilation that occurred.

Tables 1 and 2 show strikingly the increases that took place in the majority of the factors measured. The increase is not uniform on all of the days, but this probably depends upon the difficulty of maintaining a uniform condition in the period during which there was a voluntary increase in the metabolism and associated factors. In fact, on August 8 when the two periods of voluntary increase were only 5 minutes each, the rise in the oxygen absorption was not so great as on August 1 when the periods were 10 minutes long. The basal metabolism varied −9.0 to −10.9 per cent from the Harris-Benedict prediction in the normal periods and 0 to +24.9 per cent in the periods of voluntary increase. On August 8, in order to be sure that periods of slight drowsiness had not vitiated the earlier measurements on basal metabolism, we used a periodic signal, that is, an electric bell, to which the subject responded and a record of this was made by means of a signal magnet on kymograph paper. The first two normal periods on August 8 are not significantly different from those of the previous three days. The respiratory quotients on August 8 are somewhat more uniform than on the previous days and the change in the quotient is not significant on this day as compared with the marked changes that occurred during the period of voluntary increase on the other days.

Although we were not able to detect when the changes in oxygen absorption took place or how soon the oxygen absorption began to increase, we have some evidence upon the rapidity of the changes in the pulse rate and the blood pressure. For example, on July 18 the pulse as recorded from the wrist rose from 58 in the last count of the second period to 66 during the second minute after the beginning of the third period and the highest rate during that period was 70. On August 8 it rose from 53 during the last count in the normal period, by the cardiotachometer to 64 during the first quarter minute of the period of increase. The next quarter minute it was 72. In the second five minute period of voluntary increase it rose from 57 to 64 in the first quarter minute and dropped at the end of the period from 68 in the last quarter minute to 60 in the first quarter minute of the next normal period. The pulse rate changes therefore were exceedingly rapid and sharp, and there was no apparent tapering off or gradual rise either at the beginning or at the end of the period of increased metabolism.

The blood pressure was taken usually every 2 to 3 minutes during the period. On July 18 the systolic pressure rose from 114 mm. in the last observation during the second normal period to 134 mm. during the third minute after the beginning of the period of increase. The maximum in

that period was 148 mm. The diastolic pressure rose from 74 to 94 and subsequently to 98, and both of the factors fell rapidly at the end of the period. On August 1 the changes were not so abrupt as the systolic pressure rose from 104 to 126 and the diastolic pressure from 90 to 98 mm. The systolic pressure remained during four observations between 134 and 138.

The observer (F. A. H.) who took the pulse counts from the wrist and made the measurements of blood pressure stated that during the normal periods the relaxation was apparently very good but the rise in blood pressure and pulse rate that invariably accompanied the beginning of the experimental periods seemed to be almost instantaneous. On one or two occasions the signal indicating the beginning of the experimental period was given to the subject while the observer was in the midst of making a blood pressure determination. At such times considerable difficulty was noted in securing satisfactory readings, as the blood pressure changed during the observations. In the case of the pulse rate the changes were even more sudden. Several times when the rate was being counted at either the beginning or the end of a period noticeable changes in the rate were observed.

In the first two experiments, in which the subject lay with a blanket over him, it was not possible to detect the slightest change in any part of his body or any movements during either the normal periods or the periods of increased metabolism nor any change of facial expression to suggest effort or tension. On August 1 and 8 he lay without a blanket so that practically the entire body could be observed. The room temperatures were 29.5 and 26.5° C. respectively on these two days. During the first period of increased metabolism we were unable to see any actual movements such as might be due to flexing of the muscles or clenching of the hands. There was, however, at times a transverse or sidewise wave of fine movements (fibrillary) of the abdominal muscles and this took place four or five times during the 10-minute period. In the last period when the subject purposely allowed contraction of the muscles (flexors and extensors together) there were very few visible movements. There was a slight twitching or movement down the leg at one time and some apparent shifting of the abdominal muscles. The subject stated that so much tension occurred during this period that the arm muscles ached after he had finished. There was, however, no visible change in the conformation of either the leg muscles or the arm muscles. The observer of the pulse and blood pressure stated that on July 25 there were muscular tremors in the arms several times during the period of accelerated metabolism, and on August 1 there was noted a tightening of the muscles of the wrist. He said that the flesh of the arm felt more rigid and the cords in the wrist were more taut than during the control periods. This led him to believe that

the changes noted were the results of increased muscle-tonus. The subject was conscious of wide differences in skeletal muscle tonus during various accelerated periods and believed that the degree of such tonus was largely independent of the internal effort by which the increases in pulse rate and blood pressure were induced.

Although it may be considered that the changes were in part ascribable to static work, it must be noted that if all the observations had been made in the usual way with the subject either clothed or covered with a blanket, it would have been impossible to have detected any changes and the observations would have been considered as having been made under true "basal" conditions. The increases in all the factors measured were so constant and so sustained that in spite of repeated controls and observations during the periods of increased metabolism one would not have been led to look for any abnormal condition. The experiments show definitely that noticeable and significant increases in the metabolism and in the physiological processes can be brought on and sustained for periods as long as would be used in ordinary measurements of basal metabolism.

From the foregoing evidence it follows that the clinician who accepts at face value a report of a laboratory technician that the "basal metabolic rate" was such and such a figure almost invites self-deception. In a measure he can avoid being misled if he considers the pulse rate that obtained while the "basal rate" was being measured. In psychotic subjects, however, even this criterion often fails. It has frequently been observed (by R. G. H.) that in two consecutive "runs" on schizophrenic subjects the lower oxygen consumption rate may be associated with the higher pulse rate and vice-versa.

How the augmentation of these various "involuntary" processes is induced is difficult to explain. The subject had learned to detect a sensation of "tension" localized in the cardial region as an accompaniment of emotion. Having learned to recognize this "local sign," he is able to induce it at will. It may be emphasized, however, that the procedure is absolutely independent of affect (emotion). It is *not* induced by thinking of appropriately stimulating conditions or situations. It is probably no more—and no less—mysterious than is, for example, the flexing of a biceps muscle. Introspectively the two procedures seem similar. One simply "wills" the change, and it takes place. A rather striking phenomenon associated with the experiments was a subjective sense of rather marked fatigue at their conclusion. This possibly may have been due to difficulty of "forcing" nerve impulses into unaccustomed channels.

Although the experimental periods were devoid of affect, it is probable that the same mechanisms were employed as are customarily utilized in the expression of emotion. A similar state of affairs is seen when, by the injection of adrenin, a picture of sham emotion is set up. Commonly in

such cases the report of the subject is that he experienced no more than a certain degree of bewilderment at the bodily changes—of which he was conscious—but certainly no acute emotion. The significance of such observations in relation to the theory that emotion can be equated with visceral tension is obvious.

That persons with well-developed self-control can go through emotional experiences without objective disclosure of them is common knowledge. From the foregoing considerations it follows that not only technicians but investigators also should be chary of regarding any "basal metabolism" test as valid until concealed emotional tension is ruled out as a contributory factor to the oxygen consumption rate. It would seem that clear thinking would be subserved by a routine careful distinction between the customary, the episodic, and the basal metabolic rates. For some purposes one and for others, another datum is the most significant. They are not interchangeable.

SUMMARY

The total respiratory exchange, pulse and respiration rates, and systolic and diastolic blood pressures were determined with a human subject in the typical "basal" post-absorptive condition and in 5- and 10-minute periods during which he voluntarily produced an increase in all the factors without apparent visible effort.

The increases produced were from 13 to 32 per cent in the oxygen absorption, 17 to 26 per cent in the pulse rate, 9 to 28 per cent in the systolic pressure, and 4 to 27 per cent in the diastolic pressure. These changes were devoid of alterations of affect.

Only when he lay practically nude was it possible to detect any indication of effort on the part of the subject. Under the ordinary condition of measurement of basal metabolism the causes for the increased values would have remained obscure.

The observations demonstrate that it is possible for a person to maintain himself in a condition that is not basal, but which under the ordinary rules of measurement would be considered conforming to the usual conditions of basal metabolism measurements. The metabolic rate measured under the usual prescribed basal conditions is therefore not necessarily the basal rate.

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BASAL METABOLISM IN OLD AGE

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The work of earlier investigators has made it possible to draw, with considerable certainty, the curve which represents the relationship between age and basal metabolism. The portions of the curve for which the available data are the least convincing are those at puberty and in old age, particularly beyond the age of seventy. The work reported in this paper was undertaken for the purpose of furnishing data that would make it possible to more accurately determine the shape of the curve at the more advanced ages.

The pioneer work of Magnus-Levy and Falk (1899) clearly indicated that advancing age is accompanied by a decrease in the basal metabolism. Aub and DuBois (1917), using for subjects six old men who were inmates of the New York City Home for the Aged and Infirm, obtained results that were slightly higher than those of Magnus-Levy and Falk, but still well below the established normals for men of twenty to forty years of age. The relatively high figures reported by Legrand (1926), obtained on six women ranging in age from 66 to 82 years, are probably of very little significance, since the author himself seems to have felt that his subjects were not well relaxed, and furthermore suggests that they may not have been in a post-absorptive condition.

Quite recently Benedict and Meyer (1932) have published a study of the basal metabolism of twenty-three elderly women, whose ages range from 66 to 86 years. All of these women who were more than 77 years old (ten in all) had a basal heat production close to 1000 calories per twenty-four hours. The authors suggest the tentative use of this figure by clinicians as the normal standard for women 78 years of age and above. Contrary to expectation, they found that those of their subjects who appeared to have the greatest vigor had the lowest metabolic rates. Finally they conclude that "none of the existing standards may be considered to predict accurately the metabolism of any individual elderly woman."

PROCEDURE. The experiments reported in this paper were carried out at Saint Anthony's Hospital in Columbus, Ohio, and all of the subjects were inmates of this institution. Saint Anthony's Hospital is classified

by the Council on Medical Education and Hospitals of the American Medical Association as a related institution, not strictly a hospital. This classification results from the fact that a large portion of the floor space is devoted to a home for the aged. All of the subjects were inmates of this portion of the Hospital, and none of them was under routine medical treatment. They were all considered by the staff of the hospital to be normal for their ages. All of them except the oldest woman were ambulatory. Although none of the subjects engaged in regular work, most of them helped around the institution with small duties.

A total of twenty-two subjects were studied, eight women and fourteen men. The ages ranged from 74 to 106 years. One woman was restless and seemed unable to relax during the tests. Therefore the results obtained on her are not included in the averages or compared with the various prediction standards.

All determinations of basal metabolism were made with the closed circuit type of apparatus usually known as the Benedict-Roth. The subjects were connected to the apparatus by the conventional mouthpiece and noseclip. The movements of the spirometer bell were recorded by means of an automatic pen writing on a kymograph drum. From the kymograph record the oxygen consumption, total calories per hour, respiratory rate, and average tidal air were all calculated.

All tests were run between 6 and 7 a.m. The apparatus was taken to the bedside of the subject, who was given an opportunity to empty the bladder before the test. The operator was one of the authors (Matson), who at the time was an interne at the Institution, and therefore well acquainted with all of the subjects. Since the subjects were institutionalized, dietary and sleeping habits were uniform.

Typical menus were as follows: breakfast (served at 7 a.m.); bread, butter, oatmeal, eggs, coffee or milk, and fruit. Dinner (served at 12 noon); soup, bread and butter, fish or meat, potatoes and at least one other vegetable, coffee or milk, and dessert, usually consisting of pudding or cake. Supper (served at 5 p.m.); soup, bread and butter, macaroni, one vegetable, and cottage cheese. The heavy meal was always served at noon.

Pulse rate, blood pressure (both systolic and diastolic), and body temperature were taken preceding each metabolic rate determination.

Discussion of results. *Women.* In table 1 the female subjects have been listed in order of decreasing age and their most important physical characteristics tabulated. The figures which are given to the right of the ages of the subjects are the result of an effort to estimate the degree of senility of each individual. Those persons who were judged to be the least senile were given a rating of 1, while ratings of 2, 3, and 4 were used to denote increasing degrees of senility. In the succeeding columns of the

table are given the height, weight, surface area, average pulse rate, blood pressure, and tidal air. The greatest amount of variation occurs in body weight. The pulse rates and blood pressures are all within normal limits with the exception of M. D. This subject is the one already referred to as being unable to relax during her metabolism tests, who is, therefore, not included in the averages and comparisons. The pulse rate of 92, the blood pressure of 200/100, the high basal metabolism, and the extreme nervousness seem to indicate thyroid involvement and justify us in rejecting her.

The figures on tidal air, which are given in the final column of table 1, show that these women were decidedly shallow breathers. The average for all eight women is 277 cc. As would be expected, the tidal air decreases as the degree of senility increases. The women having a senility rating of 2 have an average tidal air of 314 cc., while those having a rating of 3

TABLE 1

Physical characteristics of female subjects

SUBJECT	AGE	DEGREE OF SENILITY	HEIGHT	BODY WEIGHT	BODY SURFACE	PULSE RATE	BLOOD PRESSURE	TIDAL AIR
	yrs.		*cm.*	*kgm.*	*sq.m.*		*mm.*	*cc.*
A. B.	106	4	147	31.75	1.13	66	140/80	209
R. B.	91	3	155	52.16	1.49	70	190/82	249
E. C.	84	3	163	36.29	1.31	68	170/80	254
M. N.	83	3	150	56.70	1.52	76	145/68	283
M. D.*	83		150	72.58	1.67	92	200/100	276
C. H.	80	2	150	71.67	1.66	78	180/110	321
M. B.	80	2	157	81.65	1.83	64	115/70	290
M. C.	77	2	160	68.49	1.71	70	140/70	331
Average..	85.8		154.5	56.95	1.52	70	155/08	276

* Not included in averages or comparison with standards.

have an average of but 262 cc., and A. B., the only subject with a senility rating of 4, has an average tidal air of only 209 cc. It must be pointed out, however, in this connection that the three subjects given a senility rating of 2 are the heaviest women of the group, and this fact may very well play a part in determining the tidal air.

The oldest subject, A. B., was 106 years old at the time these tests were made. She is, therefore, the oldest human being whose basal metabolism has been recorded in the literature, and her unique character as a subject justifies the following discussion. The institutional records show that she was admitted on March 10, 1925, the reason given being that on account of senility she was unable to maintain her own home. She was ambulatory until 1930, but since then she has been confined to her bed. She was born in Land, County Queens, Ireland, on October 16, 1825. Her name

is Anna Burns, her father's name was Thomas Fleming and her mother's maiden name Anna Redman. In spite of the fact that official verification is lacking, we believe that the hospital record of her birth is correct. It has been verified by two relatives who are living in Columbus.

Every effort was made to determine her exact physical condition. A complete examination of both blood and urine failed to reveal anything pathological. There is no record of her ever having had any serious illness. She has never used tobacco or alcohol. As a rule she refuses to take medicine except cathartics and occasionally sedatives. She drinks coffee once and sometimes twice a day. Considering her age her mental condition is good. She often sings old songs spontaneously, and jokes with her companions. As is customary in senility she remembers events that occurred long ago, but has no recollection of recent happenings. She sometimes becomes disoriented as to the time of day. She has never borne children and has always led an easy and comfortable life. This latter fact may, at least in part, account for her unusually long life.

On three consecutive days the food consumed by Mrs. Burns was weighed and its caloric content estimated. The averages for the three days were: total calories for twenty-four hours, 919; per cent of total calories furnished by carbohydrates, 46; per cent of total calories furnished by fat, 45; per cent of total calories furnished by protein, 9. She eats the same food as the other inmates of the institution except that as a result of the complete lack of teeth she is unable to consume food that requires mastication. The experimental data show that Mrs. Burns' basal caloric requirement is 571 calories for twenty-four hours. Her food intake is, therefore, about 60 per cent above her basal requirement, which, considering the fact that her entire time is spent in bed, would seem to be a liberal allowance.

Basal metabolism tests were run on Mrs. Burns on eight different days. Table 2, in which experimental results are recorded, shows that total calories per hour range from a low value of 21.95, obtained on April 5, to a high value of 26.25 calories, obtained on September 22. The average for the entire series is 23.81 calories, the standard deviation 1.50 calories, and the probable error ± 1.01 calorie or 4.2 per cent. This is a degree of accuracy rarely obtained in the determination of basal metabolism.

The surface area of Mrs. Burns, determined by means of the DuBois linear formula, was 1.13 square meter. A slightly different figure was obtained when the usual height-weight formula was used (1.16). Assuming that 1.13 sq.m. is the more accurate figure, the basal heat production of this subject expressed as calories per hour per square meter of body surface averaged 21.16 calories. Calories per hour per kilogram of body weight averaged 0.749. It is interesting to note that the basal figure for Mrs. Burns is 43 per cent below the value suggested by Benedict and Meyer

(1932) as a tentative standard for women between the ages of 77 and 86 years (total basal heat production, 1000 calories in twenty-four hours). This extremely low figure is no doubt partially due to the fact that Mrs. Burns was bedfast. However in evaluating this factor it is well to recall

TABLE 2

Results of metabolism tests run on Mrs. Anna Burns

Age, 106 yrs. Height, 147 cm. Weight, 31.8 kilos. Body surface, 1.13 sq.m.

DATE	O_2 CONSUMED PER MINUTE	CALORIES PER HOUR			RESPIRATORY RATE	TIDAL AIR
		Total	Per sq.m.	Per kilo		
	cc.					cc.
March 25	86.1	24.92	22.05	0.784	23	207.3
28	81.5	23.60	20.88	0.742	23	196.9
29	88.7	25.67	22.71	0.807	22	201.1
April 1	79.8	23.09	20.43	0.726	21	205.2
5	75.8	21.95	19.42	0.690	20	219.7
6	77.8	22.52	19.93	0.708	20	208.1
Sept. 22	90.7	26.25	23.23	0.825	29	228.0
28	77.7	22.47	19.88	0.707	24	207.3
Average...	82.3	23.81	21.07	0.749	23	209.3

TABLE 3

Results of metabolism tests on female subjects

SUBJECT	AGE	NO. OF TESTS	CALORIES PER HOUR			DEVIATION FROM STANDARD		
			Total	Per kgm.	Per sq.m.	Harris Benedict	Aub DuBois	Dreyer
A. B.	106	8	23.99	0.76	21.25	−21.22	−29.17	−32.25
R. B.	91	3	40.02	0.77	26.87	−5.77	−13.32	−13.54
E. C.	84	2	35.49	0.98	27.09	−6.41	−15.34	−9.04
M. N.	83	3	39.70	0.70	26.12	−12.40	−18.38	−18.66
M. D.*	83	3	58.40	0.80	34.77			
C. H.	80	2	47.01	0.66	28.33	−18.93	−11.50	−14.87
M. B.	80	3	54.69	0.67	29.88	−3.00	−6.63	−7.19
M. C.	77	3	44.98	0.66	26.30	−13.37	−20.30	−17.06
Average...	86		40.84	0.74	26.55	−11.59	−16.38	−16.09

* Not included in averages or comparison with standards.

that increasing senility is accompanied by decreasing bodily activity, and in this case we have merely reached the extreme condition where the bodily activity, in the usual sense of the word, has reached an approximate zero.

In table 3 the results of the metabolism tests on all eight of the female subjects are summarized. If A. B., who has already been discussed, as

well as M. D. is omitted, the remaining six women have an average basal heat production of 43.65 calories per hour, or 1047.6 calories for twenty-four hours. This is less than five per cent above the figure suggested by Benedict and Meyer as a tentative standard for women of this age, a really remarkable agreement. All but one of these women vary less than 16 per cent from this figure (on the basis of total calories per hour the Benedict and Meyer standard would be 41.25 calories). The subject showing the greatest discrepancy is M. B., who on comparison with the Benedict and Meyer standard has a basal rate of +31.3 per cent. It is worthy of note that this subject has the greatest body weight of anyone in the group (81.7 kilos). Furthermore the data show that the three women who had a total basal heat production above this suggested figure were the three heaviest women in the group. On the other hand, the woman who had the lowest total heat production, E. C. who was 15.8 per cent below the suggested Benedict and Meyer standard, had the lowest body weight of anyone in the group (36.3 kilos). These facts suggest that body weight has a direct effect in determining the total basal heat production even in old age. This is seen even more clearly if we consider the total calories per hour per kilogram of body weight. The average for the entire group, including A.B. but again omitting M. D., is 0.74 calorie. There is only one woman who varied more than 11 per cent from this average. This is E. C. who, as already noted, weighed but 36.3 kilos. The relatively small amount of variation in the results when the heat production is figured on the basis of calories per hour per kilogram of body weight is shown by the fact that the standard deviation from the mean of 0.74 calorie is only 0.106 calorie and the probable error 0.071 calorie, which is 9.6 per cent of the average.

Calories per square meter of body surface per hour are even more constant for this group than are the calories per hour per kilogram of body weight. If A. B. and M. D. are omitted, the average for the group is 27.43 calories per hour per square meter of body surface. M. B., who had the highest value on this basis, is but 8.9 per cent above this average, while M. N., who was the lowest, was only 5 per cent below. The standard deviation is 1.31 calories and the probable error 0.88 calorie or 3.2 per cent. This is a degree of variability only about one-third as great as that found when the heat production is figured on the basis of body weight. Therefore we conclude that for the female subjects studied in this investigation whose ages ranged from 77 to 91 years the heat production is most nearly constant when computed as calories per square meter of body surface. The basal heat production on this basis for this group of six women is 27.43 calories per hour per square meter of body surface ±3.2 per cent.

The twelve oldest subjects in the data published by Benedict and Meyer (1932) form a group in which the average age is only one year less than that of the six women considered in the above calculations. A comparison of

the average figures on these two groups of elderly women is shown in table 4. The agreement is quite good, especially considering the rather wide discrepancy in body weight between the two groups. It is also interesting to note that in Benedict and Meyer's data as in ours, the variability is much less when the heat production is figured on the basis of surface area than when it is figured on the basis of body weight. In the first case the probable error is equal to about 5.4 per cent of average, while in the second case it is equal to 11.6 per cent of the average.

With our female subjects there is little evidence of a correlation between the basal heat production and the degree of senility. In fact, from a consideration of the data presented in tables 1 and 3 we must conclude that on the subject of the relationship of senility to basal metabolic rate our data are inconclusive and do not justify the postulation of any relationship.

The final three columns of table 3 show the per cent deviation of the experimental results obtained on our female subjects from the Harris-Benedict (1919), the Aub-DuBois (1917), and the Dreyer (1921) standards.

TABLE 4

Comparison of the average figures reported by Benedict and Meyer with averages reported in this paper

	NO. OF SUBJECTS	AVG. AGE	AVG. BODY WEIGHT	AVG. CALORIES PER HOUR		
				Total	Per kgm.	Per sq. m.
			kgm.			
Benedict and Meyer	12	81.5	50.1	41.87	0.87	29.7
This paper	6	82.5	61.2	43.65	0.74	27.4

All seven of the subjects used in the averages have metabolic rates well below those predicted for them by any of these standards, although the agreement with the Harris-Benedict standards is better than that with either of the others.

In the true sense of the word there is no such thing as Aub-DuBois standards for persons in the age range covered in this investigation; however, for purposes of comparison, figures for these ages have been arrived at by a process of extrapolation. DuBois (1927) has pointed out the shortcomings of such a process, but while recognizing the unreliable nature of such figures, we feel that a comparison of our figures with them may be helpful in the process of arriving at reliable standards. Since it is generally recognized that the Aub-DuBois standards are too high, even for young adults and middle aged persons, especially females, it is not surprising that our subjects show basal rates well below these standards. The use of the Krogh (1925) or the Boothby and Sandiford (1929) modification of the Aub-DuBois standards results in better agreement.

The Dreyer (1921) standards, like those of Harris and Benedict (1919), are based on an attempt to derive a mathematical formula from which it is possible to predict the basal heat production of any normal person. The comparison of our data with figures obtained by the use of this formula gives an agreement less satisfactory than that obtained with the Harris-Benedict formula, and only slightly better than that with the Aub-DuBois standards. In general, therefore, our data may be considered as strengthening the conclusion of Benedict and Meyer that "none of the existing standards may be considered to predict accurately the metabolism of any individual elderly woman."

TABLE 5

Physical characteristics of male subjects

SUBJECT	AGE	DEGREE OF SENILITY	HEIGHT	BODY WEIGHT	BODY SURFACE	PULSE RATE	BLOOD PRESSURE	TIDAL AIR
	yrs.		*cm.*	*kgm.*	*sq.m.*		*mm.*	*cc.*
J. Mc.	92	3	168	45.81	1.50	64	170/86	363
B. D.	87	1	163	65.32	1.70	64	155/80	422
B. E.	87	3	152	56.25	1.53	64	158/80	352
M. C.	86	1	157	64.41	1.66	68	130/100	539
J. M.	85	2	173	71.67	1.85	64	174/100	428
L. M.	80	1	168	71.67	1.82	68	140/60	480
W. D.	80	1	168	78.93	1.89	60	160/84	491
F. W.	80	3	157	50.35	1.48	64	145/80	350
P. R.	79	1	157	56.70	1.56	71	158/80	408
E. C.	79	1	157	52.16	1.51	68	125/80	716
F. T.	79	2	170	81.65	1.94	70	186/100	418
G. F.	77	2	165	63.50	1.70	80	190/100	428
H. V.	77	1	170	59.87	1.70	64	149/92	670
J. W.	74	1	163	48.99	1.51	70	165/98	522
Average...	81.6		163	61.95	1.67	67	158/87	470.5

Men. In table 5 the fourteen male subjects investigated in this study are listed in order of decreasing age, and their more important physical characteristics tabulated. The ages range from 92 to 74 years, the average for the group being 81.6 years. As was done in the case of the females, the degree of senility of each subject has been estimated and indicated by numbers from 1 to 3.

The pulse rates are all normal and the blood pressures are certainly not high considering the advanced ages of the subjects. With the tidal airs, as in the case of the women, there seems to be a correlation with the degree of senility. The average tidal air for the eight men given a senility rating of 1 is 531 cc. Three have a senility rating of 2 and their average tidal air is 425 cc., while the average tidal air of the three subjects given a

senility rating of 3 is 355 cc. There is but little overlapping of these groups, and therefore we feel justified in concluding that regardless of chronological age the tidal air decreases with increasing senility. This conclusion is in agreement with the findings of Myers and Cady (1924), who reported a progressive decrease in vital capacity with advancing senility.

The heat production of the male subjects under basal conditions is given in table 6. This table shows total caloric production per hour for each subject, calories per hour per kilogram of body weight, calories per hour per square meter of body surface, and in addition the deviation of the experimental figures from the generally accepted standards.

TABLE 6

Results of metabolism tests on male subjects

SUBJECT	AGE	NO. OF TESTS	CALORIES PER HOUR			DEVIATION FROM STANDARD		
			Total	Per kgm.	Per sq.m.	Harris Benedict	Aub DuBois	Dreyer
J. Mc.	92	3	46.31	1.01	30.88	+20.22	−7.85	−3.68
B. D.	87	3	50.47	0.77	29.69	+1.57	−13.94	−12.71
B. E.	87	2	39.38	0.70	25.74	−6.68	−25.39	−26.67
M. C.	86	3	50.58	0.78	30.47	+4.94	−11.68	−12.08
J. M.	85	3	61.18	0.84	33.07	+9.31	−4.14	+0.61
L. M.	80	3	53.30	0.75	29.30	−5.40	−15.07	+3.88
W. D.	80	3	58.47	0.74	30.74	−3.36	−10.90	−9.08
F. W.	80	4	36.23	0.72	24.48	−13.39	−29.04	−29.51
P. R.	79	3	45.39	0.80	29.09	−0.79	−18.05	−16.87
E. C.	79	3	49.01	0.94	32.46	+13.67	−8.56	−6.47
F. T.	79	3	64.19	0.79	33.09	+2.80	−6.79	−2.06
G. F.	77	3	50.40	0.79	29.65	−2.85	−16.48	−13.09
H. V.	77	3	53.42	0.89	31.79	+5.07	−10.45	−5.15
J. W.	74	3	46.97	0.96	31.10	+6.60	−12.39	−8.69
Average...	81.6		50.38	0.82	30.11	±6.86	−13.62	±10.75

The average basal heat production of these fourteen men figured on the basis of total calories per hour is 50.38 calories. The subject producing the fewest calories per hour was F. W. (36.23 calories). Although F. W. was neither the shortest nor the lightest man in the group, he was nevertheless the one who had the least surface area (1.48 sq. m.). The subject producing the greatest number of calories per hour was F. T. who averaged 64.19. The degree of variability in the number of total calories produced per hour by these subjects can best be indicated by stating that the standard deviation is 7.47 calories, while the probable error is 5.04 calories, an amount equivalent to ±10 per cent of the mean.

When the heat production is figured on the basis of calories per hour per

kilogram of body weight, the highest figure is that for J. Mc., the oldest man in the group. He produced 1.01 calorie per hour per kilogram of body weight. Table 5 further shows that the man having the next highest heat production on this basis is J. W., the youngest subject, who produced 0.96 calorie per hour per kilogram of body weight. The subject B. E. produced but 0.70 calorie per hour per kilogram of body weight, the lowest figure on this basis of any subject in the group. The degree of variability of heat production figured on the basis of body weight is considerably less than that found for total calories per hour. The standard deviation from the mean value for calories per hour per kilogram of body weight is 0.092 calorie, and the probable error is ± 0.062 calorie, which is equal to 7.6 per cent of the mean.

As was found to be the case with the female subjects, the heat production when figured on the basis of calories per square meter of body surface is much more constant for this group than either total calories or calories per kilogram of body weight. The average number of calories per hour per square meter of body surface is 30.11. The range is from 25.74 calories per hour per square meter of body surface for the subject B. E. to 33.09 calories per hour per square meter of body surface for F. T. The small amount of variability shown by this figure is indicated by the fact that the standard deviation is only 2.40 calories and the probable error ± 1.62 calories, which is equivalent to ± 5.4 per cent of the mean.

With the male subjects the evidence of a correlation between the degree of senility and the basal metabolic rate is even less convincing than was the case with the female subjects. The eight elderly men who were judged to be the least senile had an average total heat production of 50.95 calories per hour. On the basis of body weight they averaged 0.83 calorie per kilogram of body weight per hour, and on the basis of body surface the average for the group is 30.58 calories per square meter of body surface per hour. The corresponding averages for the three subjects placed in the intermediate group, given a senility rating of 2, are total calories per hour, 58.59; calories per hour per kilogram of body weight, 0.81; and finally calories per hour per square meter of body surface, 31.94. For the three subjects judged to be the most senile of all the men investigated (senility rating 3) the averages are 40.64 calories per hour, total heat production; 0.81 calorie per hour per kilogram of body weight; and 27.03 calories per hour per square meter of body surface.

These figures would seem to indicate that the group of men judged to be the most senile actually do have the lowest metabolic rate, although even here the calories per kilogram of body weight average exactly the same as for the group given a senility rating of 2. However the group given a senility rating of 2 are distinctly higher, both in total calories per hour and in calories per hour per square meter of body surface, than the group given

a senility rating of 1. It is true that on the basis of calories per hour per kilogram of body weight they are slightly lower than the group rated 1, but even here the difference is too slight to be significant. As in the case of the women, we must therefore conclude that our data give no clear cut indication of a correlation between the degree of senility and the basal metabolic rate, although there is some indication that those subjects who were judged to be the most senile had the lowest metabolic rate.

The failure of our data to indicate a relationship between the degree of senility and the basal metabolic rate does not, of course, disprove the existence of such a relationship. It may very well be that our method of judging senility is inaccurate, or that our data are not extensive enough to justify conclusions on this point, or that an existing relationship is masked by other uncontrolled variables.

The agreement of the experimental data with the existing normal standards is considerably better than was the case with the female subjects. As with the women the best agreement is with the Harris-Benedict (1919) standards. The average deviation from these standards is only 6.9 per cent, while only three of the fourteen deviate ten per cent or more. Furthermore the distribution of the data is almost normal, as eight of the subjects show plus deviations and six of them negative deviations. With the female subjects all deviations from all three of the standards are negative, which is strong evidence that existing standards for elderly women are too high.

When compared with figures obtained by extrapolation from the Aub-DuBois (1917) standards all of our male subjects show negative deviations, the average for the group being -13.6 per cent. Ten of the fourteen subjects show deviations greater than -10 per cent. As was pointed out with the female subjects, the use of the Krogh (1925) or Boothby and Sandiford (1929) modification of the Aub-DuBois standards would result in closer agreement with the experimental data. The average deviation from the Dreyer (1921) standards is 10.8 per cent. All but two of the subjects show negative deviations, and six of the fourteen show deviations greater than -12 per cent.

These figures seem to justify the conclusion that of generally accepted standards the Harris-Benedict prediction formula gives figures which most nearly agree with the experimentally determined basal metabolism of elderly men. The Dreyer formula and the Aub-DuBois standards give figures that are from 10 to 14 per cent above experimental results.

These data give an excellent opportunity to compare the metabolism of elderly men with that of elderly women. If A. B. and M. D. are omitted, we have a group of six women whose average age is 82.5 years, about eleven months more than the average age for the fourteen subjects that comprise our group of elderly men (81.6 years). Total calories per

hour for the women average 43.65, while the men averaged 50.38 calories per hour. On the basis of total calories per hour, therefore, the men have a metabolism which is 15.4 per cent higher than the women. On the basis of calories per hour per kilogram of body weight the average for the women is 0.74 calorie and for the men 0.82 calorie, the figure for the men being 10.8 per cent higher than that for the women. When the heat production is figured as calories per hour per square meter of body surface, the average for the women is 27.43 calories and for the men 30.11 calories. Here the men are 9.8 per cent higher than the women. These differences between the sexes are of about the same magnitude as that already established for adults of less advanced age. We are, therefore, justified in concluding that the difference in basal metabolic rate between the sexes persists in old age without change in magnitude.

SUMMARY

A series of basal metabolism tests carried out on eight women ranging in age from 77 to 106 years, and on fourteen men whose ages ranged from 74 to 92, gave the following results:

1. The oldest subject, a woman 106 years old, 147 cm. tall and weighing 31.8 kilos, had a total heat production of 23.81 calories per hour. On the basis of body weight she produced 0.749 calorie per hour per kilogram of body weight, and on the basis of body surface 21.07 calories per square meter of body surface.

2. The results on one female subject were discarded as there was some indication of thyroid dysfunction. The remaining six with an average age of 82.5 years gave the following averages: total heat production, 43.65 calories per hour; calories per hour per kilogram of body weight, 0.74; calories per hour per square meter of body surface, 27.43.

3. The fourteen men, whose average age was 81.6 years, gave the following averages: total calories per hour, 50.38; calories per hour per kilogram of body weight, 0.82; and calories per hour per square meter of body surface, 30.11.

4. The experimental results were compared with the Harris-Benedict, Aub-DuBois, and Dreyer standards. The results on the female subjects were well below all of these standards, although the best agreement was obtained in the case of the Harris-Benedict standards. With the men the agreement with the Harris-Benedict standards was fairly satisfactory, but the other two standards gave figures much higher than the experimental results.

5. With both men and women the calories per square meter of body surface were much more constant than either total calories or calories per kilogram of body weight.

6. An attempt was made to estimate the degree of senility of each of

the subjects. With neither the men nor the women was there a clear cut indication of any relationship between the degree of senility and the metabolic rate. However, there did seem to be a relationship between tidal air and senility, the tidal air decreasing as the degree of senility increased.

7. The difference between the sexes was of approximately the same magnitude as that for adults of less advanced age. On the basis of total calories the men were 15.4 per cent higher than the women, on the basis of body weight the difference was 10.8 per cent and on the basis of surface area 9.8 per cent.

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THE LIPID COMPONENTS OF THE LYMPH OF THE THORACIC DUCT OF THE DOG[1]

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About half of the cholesterol taken in the food is readily absorbed from the intestinal tract, passes through the thoracic duct (Schoenheimer, 1931) and appears in the blood as free and esterified cholesterol (Dorée and Gardner, 1909; Fraser and Gardner, 1909 and 1910; Mueller, 1915). It is excreted in the bile, but a portion is reabsorbed (Dorée and Gardner, 1908). Although there is considerable evidence that the body is able to synthesize cholesterol (Dezani, 1914; Gamble and Blackfan, 1920; Channon, 1925; Randles and Knudson, 1925; Schoenheimer, 1931 and 1933), every attempt is made to conserve its supply. Its function in the bile is not entirely known, but its reabsorption suggests that, like the bile salts, it may be of some aid in the absorption of fat.

It is the chief purpose of this investigation to study the rôle cholesterol plays in fat metabolism. The effect of adrenaline on the total fatty acids, glucose and lactic acid of the thoracic duct lymph and the carriage of fat by the portal vein were also studied.

METHOD. Observations were made on 24 dogs, 3 to 8 days post-absorptive and weighing from 15 to 30 kgm. Four served as controls, 10 received a fat meal and 10 other animals, 1 phlorhizinized, 2 depancreatized and 7 normal, were given injections of adrenaline. All were anesthetized with sodium amytal given intraperitoneally. The thoracic duct was cannulated and the femoral artery exposed. Seven fat-fed dogs had the portal vein exposed. The lymph was collected under mineral oil in a centrifuge tube, and the clear fluid was pressed out with a glass rod from the clotted lymph. In order to analyze the serum, whole blood was delivered under oil into a centrifuge tube and centrifuged for 30 minutes. In some of the experiments sodium oxalate was added to the blood to obtain plasma.

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The experimental data in this paper are taken from the dissertation submitted by Susan H. Brockett in partial fulfillment of the requirement for the degree of Master of Science, Yale University, June, 1934.

The serum and lymph were analyzed for total fatty acids (Man and Gildea, 1932), lipoid phosphorus, free and total cholesterol (Man and Peters, 1933), glucose (Hagedorn and Jenssen, 1923), and lactic acid (Friedemann, Cotonio and Shaffer, 1927).

RESULTS. The data presented in table 1 are typical of four experiments. Although the lipid components of serum are quite constant in the post-

TABLE 1

The lipid content of thoracic lymph and serum of normal, fasted and amytalized dogs*

Male, fasted 5 days, weighing 22.4 kgm.

FLUIDS ANALYZED	TIME	TOTAL SOLIDS	TOTAL FATTY ACIDS	LIPOID PHOSPHORUS	FREE CHOLESTEROL	TOTAL CHOLESTEROL
		mgm./ 100 cc.	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*
Lymph	9:45–11:00	65	451	12	45	190
Serum	11:00	86	303	13	43	191
Lymph	11:00–1:15	62	310	9	44	191
Serum	1:15	86	303	13	45	192
Lymph	1:15–3:15	57	281	6	23	75

* The free cholesterol and total cholesterol as well as the total fatty acids in this table and table 2 have been corrected for changes in concentration.

TABLE 2

The effect of feeding fat on the lipid content of the thoracic lymph

Female, fasted 8 days, weighing 30 kilos. Fed 100 grams soy bean oil by stomach tube 9:00 A.M.

FLUIDS ANALYZED	TIME	TOTAL SOLIDS	TOTAL FATTY ACIDS	LIPOID PHOSPHORUS	FREE CHOLESTEROL	TOTAL CHOLESTEROL
		mgm./ 100 cc.	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*
Lymph	10:30–12:50	115	1,705	13	70	376
Serum	12:50	91	414	11	48	191
Lymph	12:50–3:45	110	2,274	17	83	629
Serum	3:45	98	385	11	49	192
Lymph	3:45–9:00	86	1,360	16	91	251

absorptive state, this is not true in the lymph where they gradually decrease with dilution.

The data presented in table 2 are representative of three experiments. The feeding of soy bean oil brought about striking changes in the lipid constituents of the thoracic lymph. With the marked rise and gradual fall in total fatty acids there is a slight but simultaneous rise and fall of the lipoid phosphorus and a similar though more marked variation of the total

cholesterol (free and cholesterol esters). The increase in free cholesterol parallels the rise but not the fall of the fatty acids.

Table 3 gives data illustrative of seven experiments. The ingestion of fats, which caused a very marked increase in fatty acid content of the thoracic lymph did not alter the fat composition of the portal vein. Tying off the thoracic duct did not bring about any change in the fatty acids of the portal vein.

Table 4 gives the data of two of the ten experiments on seven normal, one phlorhizinized and two depancreatized dogs into which adrenaline was injected in an attempt to determine the effect on the total fatty acid, glucose and lactic acid content of the thoracic lymph. With the decrease in concentration of total solids there was a steady fall in total fatty acids,

TABLE 3

Analysis of thoracic lymph, arterial and portal serum for total fatty acids after feeding fat

Female, fasted 4 days, weighing 18 kilos, fed ¼ lb. butter by mouth 8:12 (some vomiting)

TIME	LYMPH		FEMORAL ARTERY		PORTAL VEIN	
	Total solids	Total fatty acids	Total solids	Total fatty acids	Total solids	Total fatty acids
	mgm./100 cc.	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 cc.*
9:00			99	347	100	351
10:10–10:40	84	886				
10:40–11:10	88	1,179				
11:10–11:30	85	1,372				
11:30–11:55	89	1,463				
11:55			100	346	103	322
11:55–1:50	87	1,037				
1:50–3:50	80	649				
3:50			100	350	103	336

an effect unaltered by the injection of adrenaline. The adrenaline caused an increase in glucose and lactic acid not only in the plasma and serum but also in the lymph.

Discussion. The lipid components of serum in dogs are constant in a postabsorptive condition. The lipoid phosphorus range in all of the experiments is between 11 and 13 mgm. per 100 cc. of serum. The free cholesterol and total cholesterol showed a greater variance. One hundred cubic centimeters of serum had from 48 to 67 mgm. of free cholesterol and from 195 to 240 mgm. of total cholesterol. Where it was possible to correct for variations in concentration it was noted that there was very little change in either free or total cholesterol in a given dog, and that 25 to 33 per cent of the total cholesterol was in the form of free cholesterol.

In the lymph of fasted dogs, the total fatty acids decrease with the dilution of lymph. This change is much more marked in some dogs than in others. Adrenaline, even in very large doses, had no apparent effect on the total fatty acid curve which confirms the results of Rony, Mortimer and Ivy (1933). With this decrease of fatty acids there is a corresponding change in the lipoid phosphorus and total cholesterol. In an attempt to analyze this relationship further a pure fat was fed to dogs. The total

TABLE 4

The effect of injections of adrenaline on the total fatty acid, glucose and lactic acid content of the thoracic lymph of dogs

FLUIDS ANALYZED	TIME	TOTAL SOLIDS	TOTAL FATTY ACIDS	SUGAR	LACTIC ACID
Female, fasted 3 days, weighing 17.7 kgm.					
		mgm./ 100 cc.	*mgm./ 100 cc.*	*mgm./ 100 cc.*	*mgm./ 100 cc.*
Lymph.	9:50–10:40	59	376	152	
Serum	10:35		324	153	
6 cc. adrenaline subcutaneously 1 cc. every 15 minutes					
Lymph	10:40–11:11	56	383	152	
Lymph	11:11–11:40	56	324	174	
Lymph	11:40–12:16	57	291	253	
Serum	12:20			278	
Lymph	12:16–12:59	56	260	323	
Lymph	12:59–1:44	54	259	416	
Lymph	1:44–2:30	53	259	422	
Lymph	2:30–3:17	53	240	438	
Male, fasted 8 days, weighing 22 kgm.					
Plasma	2:26	97	379	97	29
Lymph	2:25–3:30	50	294	108	17
8 cc. adrenaline subcutaneously 1 cc. every 15 minutes					
Lymph	3:30–4:45	53	248	121	22
Plasma	4:50	95	398	118	29
Lymph	4:45–5:57	51	220	130	26
Lymph	5:57–8:00	52	221	177	72
Plasma	8:30	95	398	234	108

fatty acids in the thoracic lymph first increased and finally fell as absorption was completed. The lipoid phosphorus and total cholesterol parallel the rise and fall of the fatty acids, but this does not occur with the free cholesterol as this substance is still increasing when the fatty acids have already begun to decrease as if the bile secretion has not ceased with the absorption of most of the fat. This and the fact that there is such a small rise of lipoid phosphorus in comparison with the total cholesterol indicate

that the cholesterol esters rather than the lipoid phosphorus are principally involved in the transport of fatty acids.

In a dog fed a pure oil, one must look elsewhere than the diet to account for the sudden increase in cholesterol. The bile, formed by the liver, is probably the source of this cholesterol. However, bile contains only free cholesterol, no cholesterol esters (Thannhauser, 1923). Thus the esterification of the cholesterol must occur either in the lumen or wall of the intestine or in the thoracic duct. Mueller (1916) has reported an analysis of the intestinal mucosa which revealed that the cholesterol which had been absorbed from the intestine had already been largely esterified by the time it reached or at least before it left the lymphatics of the mucosa. He concluded that the change probably takes place slowly in the lumen of the intestine.

The peak of the increase in the lipid substances of the thoracic duct occurs about the sixth to eighth hour, the same time noted by Bloor (1915) as the peak of lipemia. Since there is a rise and fall in total cholesterol which parallels that of the total fatty acids, the changes in cholesterol, though secondary as suggested by Bloor (1921), Iscovesco (1912) and Knudson (1917), follow so closely as to be practically simultaneous with the rise in total fatty acids. It would appear that this constituent of the bile has as one of its important functions the facilitation of the absorption of fats by forming esters with the fatty acids, probably in the lumen of the intestine, carrying them across the intestinal wall and through the thoracic duct to the general circulation.

However, only about 60 per cent of the absorbed fat can be accounted for in the chyle. Bloor (1922) suggested that the other 40 per cent might be carried by the portal system. d'Errico (1907) and Cantoni (1928) believe that the fatty acid content of the portal blood is always higher than that of the general circulation after a meal of fat, though Zucker (1919) reported negative results. The observations here recorded agree with those of Zucker as at no time during the course of the experiments did the total fatty acid concentration of the serum of the portal vein differ to any marked degree from that of the serum of the femoral artery.

The increase of phosphatides in the thoracic lymph after the ingestion of fat indicates that the phosphatides are involved in the transport of fats. Since the phosphatides are miscible in water it is possible that the portal system may carry the end products of fat digestion in this form. This might explain how the remaining 40 per cent of the fat is transported. However a consideration of the right thoracic duct might be of greater significance in this connection (Drinker and Field, 1933).

The rise of lactic acid and glucose in the thoracic duct following injections of adrenaline are due in part to the lactic acid poured directly into the muscle lymphatics and the glucose into those of the liver. However, these

diffusible substances may pass from the capillary beds of the entire body into the extra-vascular fluids and thus finally appear in the thoracic duct.

SUMMARY AND CONCLUSIONS

1. The lipid content and the relative proportions of free and total cholesterol of the serum are constant in the post-absorptive state.

2. The concentration of the lipid constituents of thoracic lymph are dependent upon the total fatty acids present. After feeding fat there is a great increase of total fatty acids, a moderate one of lipoid phosphorus and a marked rise of total cholesterol (free and cholesterol esters). It is concluded that cholesterol aids in the absorption of fatty acids.

3. The portal vein does not appear to carry absorbed fatty acids from the intestine to the liver during digestion.

4. Injections of adrenaline bring about no changes in the total fatty acid content of the thoracic lymph, but do cause a rise in glucose and lactic acid in the lymph.

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THE METABOLISM OF TISSUES EXCISED FROM ADRENALECTOMIZED RATS[1]

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Britton and Silvette (1934) have demonstrated that adrenalectomy causes marked changes of carbohydrate metabolism consisting of a lowering of the level of blood sugar and a depletion of the glycogen reserves. The present communication is concerned with the effect of glucose on the respiratory metabolism of tissues excised from adrenalectomized rats.

METHODS. Rats were subjected to double adrenalectomy under nembutal anesthesia. These animals as well as normal controls ingested a mixed diet to which sodium chloride was added. Despite some loss of weight, the operated animals retained their appetite until premortal symptoms supervened. These include marked muscular weakness, occasionally diarrhea and more rarely convulsions. The technique developed by Warburg was employed to determine the gaseous exchanges of tissues excised from the adrenalectomized rats and from the normal controls. Cerebral cortex was minced, kidney and liver sliced, and diaphragm sectioned. The seminiferous tubules were obtaining by cutting the testicular coats. The suspending medium, buffered at pH 7.4 with phosphate, was Ringer's solution except in the case of diaphragmatic muscle for which isotonic saline was used.

RESULTS. The averages of the results presented in table 1 reveal a decrease of the oxygen utilization of the cerebral cortex. This, as well as the diminished respiratory quotient, are noted during extremis when the cerebral cortex is studied without glucose in the suspending medium. In the presence of glucose, the respiratory quotient rises but the oxygen consumption is nevertheless lower than that of the normal controls. The observations of the adrenalectomized rats still apparently in good condition not included in the table are not significantly different from those of the control animals. The oxygen consumption of the testicle of the adrenalectomized rats is depressed in the absence of glucose though the respiratory quotient remains unaltered. The liver of the operated animals exhibits an oxygen uptake decreased not beyond the error of the method

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while the respiratory metabolism of kidney and diaphragm are unaffected by adrenalectomy.

DISCUSSION. The respiratory metabolism of cerebral cortex excised from rats in acute suprarenal insufficiency exhibits marked changes including a depression of the oxygen utilization. The decrease of the respiratory quotient in the absence of glucose, moreover, indicates a change in the character of oxidations. One experiment was not included in the table because a concentration of glucose of only 65 mgm. per cent was used in the Warburg chamber, the same concentration as had obtained in the animal just previous to the removal of the brain. This small

TABLE 1

Respiratory metabolism of excised rat tissues

ORGAN	WEIGHT OF TISSUE	EXTREMIS				CONTROLS			
		Number of observations	Substrate	R.Q.	O_2, mm^3/hr.	Number of observations	Substrate	R.Q.	O_2, mm^3/hr.
	mgm.								
Brain	125	10	None	73	45	9	None	94	134
		8	Glucose	91	139	7	Glucose	92	199
Testicle	200	7	None	79	68	7	None	78	120
		6	Glucose	91	141	7	Glucose	88	172
Liver	200	11	None	66	107	8	None	61	138
		10	Glucose	67	104	9	Glucose	62	134
Kidney	60	8	None	73	135	8	None	74	146
		8	Glucose	77	173	8	Glucose	76	182
Diaphragm	150	8	None	82	117	11	None	81	118
		8	Glucose	82	111	11	Glucose	87	126

concentration of glucose produced a rise of the oxygen uptake from an original value of 43 $mm.^3$ to 87 $mm.^3$, the latter being lower than the smallest oxygen utilization of the cortex of the control animals without the simulating effect of glucose. This emphasizes the fact presented in the table that in the presence of glucose the oxygen consumed by the brain of animals in extremis is low in comparison with that of the controls with the same substrate. Thus the depressed metabolism of the brain is probably an integral part of the syndrome of adrenalectomized animals in extremis.

In an effort to determine the mechanism of the production of the altered brain metabolism, the spinal cord of normal rats was transected just below

the brachial plexus from 3 to 18 hours before excision of the tissues. In this manner the effects of low blood pressure and shock could be determined. To intensify the condition of shock, the animals were also subjected to hemorrhage. Four experiments revealed changes in the gaseous exchange of the brain similar to those occurring in the adrenalectomized animals, i.e., in 2 experiments the respiratory quotients were 0.76 and 0.77 and the oxygen uptake 83 mm.^{3} and 86 mm.^{3} respectively. That the central nervous system is specially sensitive to low blood pressure has been emphasized by Cannon and Cattell (1932). These authors review the literature and present the evidence for functional and morphologic changes in the brain due to insufficient blood supply. The depressed metabolism of the brain is therefore an expression of a fundamental change which is not peculiar to adrenalectomized animals in extremis but may occur in any condition involving a continued low blood pressure and shock. Apparently the maintenance of cerebral integrity necessitates a large and continuous supply of energy from blood glucose derived anaerobically (Nahum and Himwich, 1931) and aerobically (Holmes and Holmes, 1925) and from the oxygen obtained of blood. Even with ample amounts of glucose (table 1), the changes of the brain are not entirely reversible, i.e., an O_2 uptake in the presence of glucose of 139 mm.^{3} in the operated animals vs. 199 mm.^{3} in the controls, suggests that, after a certain period of depressed oxidations, treatment which may restore many of the organ systems of the body will nevertheless not be effective for the central nervous system. If this is the case, then the alterations of the brain may be the cause of the failure of treatment occasionally occurring in adrenalectomized animals.

Only the brain disclosed significant changes of carbohydrate metabolism, but even the brain is capable of oxidizing added glucose. The impaired cerebral oxidations are, therefore, not caused by inability to oxidize carbohydrate. It is true that the oxygen consumption of the testicle was diminished in adrenalectomized rats. The respiratory quotient, however, remained unchanged, indicating that the various foodstuffs were oxidized in the same proportions. The liver and diaphragm both of the control and operated animals failed to respond to the glucose in the suspending medium though the respiratory quotient of the diaphragm yielded evidence for the combustion of similar amounts of carbohydrate in both groups of animals. Glucose had the same stimulating effect on the gaseous exchange of the kidney of the normal animals and those in cortical adrenal insufficiency.

SUMMARY AND CONCLUSIONS

Numerous observations have been made with the aid of the Warburg technique of the respiratory metabolism of organs: brain, testicle, diaphragm, liver, kidney, excised from adrenalectomized rats, both in ex-

tremis and apparently in good condition as well as of control animals. The respiratory metabolism of the liver, kidney and diaphragm was unaltered by adrenalectomy. The testicle and the brain, however, exhibited a depressed oxygen consumption in the absence of glucose. The brain, moreover, suffered a decrease of respiratory quotient. In the presence of glucose, the oxygen uptake of cerebral cortex of the rats in extremis did not rise to the level of that of the normal controls with the substrate. This cannot be imputed to inability of the brain to oxidize carbohydrate. The present observations of the various excised tissues disclose no such inability.

Changes of cerebral metabolism similar to those produced by adrenalectomy also occurred in rats subjected to transection of the cord and hemorrhage over a period of several hours.

It is concluded that depression of metabolism is produced by lack of energy obtained from the blood constituents, glucose and O_2. This depression becomes an integral part of the syndrome of acute adrenal insufficiency. The results indicate, moreover, that the cerebral changes are, to a certain extent, irreversible and may be a cause of failure of treatment. Furthermore, the depressed metabolism is not peculiar to acute cortical adrenal insufficiency, but may occur in any conditions involving low blood pressure over a period of hours.

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THE RESPIRATORY QUOTIENT OF MUSCLE OF DEPANCREATIZED DOGS[1]

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Several years ago a study was made in this laboratory of the respiratory quotient of intact voluntary muscle of depancreatized and of phlorhizinized dogs under amytal anesthesia. Since the results were not published, it may be well to summarize them at this time. The respiratory quotient of resting muscle of depancreatized dogs was determined 6 times. However, most observations were made on activated muscles stimulated directly once per second with an induction coil. Such quotients were obtained 15 times on depancreatized dogs, and 15 were also taken on phlorhizinized animals. In 3 experiments on the phlorhizinized dogs, and one on a depancreatized dog, the respiratory quotients were below 0.7, and in the remaining experiments they varied from 0.72 to 1.01. Five observations on activated muscle revealed no changes in the CO_2 and O_2 capacities of the blood samples. In 8 out of 13 experiments the muscles were found to add lactic acid to the blood, and only twice was lactic acid removed from the blood stream. In the present study an attempt was made to determine the factors which produce these variations in the respiratory quotient of diabetic muscle.

METHOD. Forty-eight to seventy-two hours after the aspetic removal of the pancreas, blood samples were obtained from eleven unanesthetized, quietly resting dogs. The blood was drawn by direct puncture through the skin practically simultaneously from the femoral artery and femoral vein, and was kept over mercury in glass tonometers which contained powdered sodium fluoride and potassium oxalate. Observations of the CO_2 and O_2 content (1), and the total acetone substances (2) were obtained on every blood sample. The error of the acetone method was $\pm$ 0.8 mgm. per cent and differences of 2.4 mgm. per cent were considered significant. In some instances, the pH (3), CO_2 and O_2 capacities (1) and lactic acid (5) were determined.

RESULTS. The sixteen observations obtained on the 11 dogs are pre-

[1] Aided by a grant from the Research Fund of Yale University School of Medicine.

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sented in table 1 in order of ascending respiratory quotients. They varied from 0.50 to 1.24. We are unable to offer any explanation for the single abnormally high respiratory quotient found in these experiments. Of the 15 remaining observations, 3 respiratory quotients were between 0.50 and 0.65, and 12 quotients were between 0.73 and 0.99.

The CO_2 capacity of the simultaneously drawn arterial and venous samples was in agreement within the experimental error (1 vol. per cent) in 5 of the 7 experiments, and the O_2 capacity was unchanged in 6 of the same 7 experiments.

TABLE 1

Constituents of arterial and venous blood of lower extremity

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
DATE	CO_2 CAPACITY		O_2 CAPACITY		CO_2 CONTENT		O_2 CONTENT		R.Q.	ACETONE			LACTIC ACID		pH	
	A	V	A	V	A	V	A	V		A	V	Balance	A	V	A	V
1933																
5/19					39.0	42.6	16.7	9.4	0.50	5.1	12.0	−6.9			7.23	7.12
2/1					20.5	29.4	22.3	8.0	0.63	0	24.0	−24.0			7.08	7.01
6/14	30.3	29.4	20.5	20.8	33.0	38.2	17.9	10.0	0.65	8.6	17.2	−8.6				
5/5					33.7	36.9	17.5	13.0	0.73	28.6	24.6	+4.0			7.21	7.19
1/25					29.1	41.8	26.1	8.8	0.74	7.2	6.7	+0.5			7.23	
7/6	36.7	37.8	23.8	23.4	41.0	46.9	19.9	12.2	0.76	8.0	2.0	+6.0	24	17	7.35	7.30
1/11					23.9	37.3	19.6	2.8	0.80	17.8	7.7	+10.1			7.15	6.96
2/1					18.5	31.5	22.5	6.4	0.80	10.0	0	+10.0			7.08	7.00
2/27	39.8	39.9	20.7	20.7	43.3	46.9	15.6	11.2	0.81	8.2	0	+8.2			7.31	7.27
7/6	37.9	39.9	23.6	20.9	41.3	45.9	19.7	14.3	0.85	6.0	3.0	+3.0	23	17	7.35	7.34
7/12	27.3	26.8	24.8	25.3	26.3	34.8	21.3	11.4	0.87	10.0	4.5	+5.5	19	22		
5/25					45.1	52.2	24.4	16.6	0.90	11.8	4.1	+7.7				
7/12	27.8		24.2		26.0	37.8	21.0	8.3	0.93	7.5	10.0	−2.5	13	22		
6/21	20.0	19.9	30.8	30.5	18.6	29.2	25.6	14.5	0.95	10.0	3.0	+7.0	68	74		
6/21	20.3	14.5	30.1	30.1	18.9	26.2	25.3	17.9	0.99	24.0	18.0	+6.0	52	49		
2/27	38.2	38.1	20.8	21.1	41.6	52.1	17.7	8.7	1.24	1.0	8.0	−7.0			7.31	7.20

Blood gases in volumes percent. Acetone and lactic acid in milligrams percent.

The pH of the venous blood was lower than that of the arterial sample in each of the 8 experiments in which analyses were made. The differences varied from 0.01 to 0.19.

The lactic acid balance of the muscle was determined in 6 instances. On two occasions the muscle was found to remove lactic acid from the blood; lactic acid was added to the blood twice; and in 2 experiments the differences between the arterial and venous concentrations were not significant, being less than 5 mgm. per cent.

In each of the 3 experiments in which the respiratory quotients were

below 0.7, the muscle liberated acetone substances. In contrast to these results, in the 12 experiments with respiratory quotients above 0.7, the acetone was absorbed 10 times and liberated once.

Discussion. The true respiratory quotient obtained by analyses of simultaneously drawn arterial and venous blood samples is determined by the character of the foodstuffs oxidized in the muscle. However, the apparent respiratory quotient may differ from the true quotient if changes of the acid-base equilibrium or concentration of the blood occur while passing through the muscle. In the present experiments, the CO_2 and O_2 capacities and pH of the blood were analyzed in order to determine whether the variations of the respiratory quotient were due to such non-oxidative changes. There was no significant difference in the CO_2 capacity of the blood as a result of passage through the muscle, and although there was a fall in pH it was independent of the level of the quotient, the acetone or lactic acid exchanges. These lines of evidence, therefore, indicate that in the present experiments the variations in the respiratory quotient were not affected in any great measure by disturbances of the acid base equilibrium. The concentration of the blood, as indicated by the O_2 capacity, was unchanged by passage through the muscle in almost all cases. Such a constancy of the acid base and water equilibria of the blood might be expected under experimental conditions in which the dogs were unanesthetized and resting quietly. It is therefore probable that the quotients observed are valid. In the following discussion an effort will be made to estimate the character of the oxidative reactions which might explain the variations found in the respiratory quotient.

The chief foodstuff oxidized during diabetes is fat with a respiratory quotient of 0.7. The incomplete combustion of fatty acids, represented by palmitic acid, with the liberation of diacetic acid results in a respiratory quotient of 0.63. It may therefore be significant that the 3 quotients observed below 0.7 were associated with a liberation of acetone by the muscle. Of these low respiratory quotients, those of 0.65 and 0.63 may be the result of such an incomplete combustion. The quotient of 0.50, if not due to experimental error, may be the result of the incomplete combustion of fatty acids with chains shorter than 16 carbons. In the 12 observations with quotients above 0.7, acetone substances were absorbed 10 times and liberated once. The respiratory quotients of acetone, betahydroxybutyric acid and diacetic acid are 0.75, 0.89 and 1.0 respectively. The experiments in which the respiratory quotient was above 0.7 may therefore be due to the oxidation of a mixture of fat and ketone acids. In a previous work (6) it was suggested that "it is also possible that the ketone acids which were removed were oxidized; and those cases in which the organs added acetone substances to the blood might be due to the incomplete oxidation of fatty acids." The correlation of the quotients below 0.7 with the liberation

of ketone substances, and especially that of the high quotients with the absorption of these substances, suggests that the liberation and absorption of ketone acids is the result of oxidative reactions. This correlation was obtained even when 2 successive respiratory quotients in the same animal (2/1 1933) were above and below 0.7 respectively.

It is well known that 48 hours after pancreatectomy, the dog has a diminished ability, if not a total inability, to oxidize glucose. Shorr, Loebel and Richardson (7), however, have demonstrated that in vitro diabetic muscle tissue can oxidize lactic acid. The respiratory quotients above 0.7 may therefore represent the oxidation of a mixture of fat with small amounts of carbohydrate, lactic acid or ketone substances, or a mixture of these. If lactic acid is oxidized in vivo by the depancreatized muscle, it may supply the antiketogenic substances required for the oxidation of ketone acids.

Forty-eight to seventy-two hours after pancreatectomy, the respiratory quotient of the entire animal approximates a ratio of 0.7. Since the present observations indicate that muscle may have a quotient above that value and it is known that the respiratory quotient of the brain is fixed at unity (8), some parts of the body must have quotients below 0.7. The respiratory quotient of the liver of fasted rats studied in vitro is frequently low, Meyerhof and Lohmann (9) reporting, in some instances, values of 0.45. Certainly during diabetes, the liver is constantly releasing acetone substances (6) and therefore probably has a low quotient in vivo. Thus, the respiratory quotient of 0.7 found for the entire animal represents the resultant of the quotients of the various organs. It has already been suggested by Cathcart and Markowitz (10) that the respiratory quotient indicates the sum total of the metabolic processes occurring in the body.

SUMMARY AND CONCLUSIONS

Analyses were made of 16 pairs of blood samples drawn simultaneously from the femoral artery and femoral vein of 11 unanesthetized, quietly resting depancreatized dogs. The respiratory quotient, CO_2 and O_2 capacities, acetone substances, lactic acid, and pH were determined. In 3 experiments, the quotients were below 0.7, and in 12, were above that value. These variations of the respiratory quotient could not be accounted for by changes in the acid base balance or concentration of the blood. In each of the experiments in which the respiratory quotient was below 0.7, acetone substances were liberated into the blood stream; while in 10 of the 12 experiments in which the respiratory quotients were above 0.7, acetone substances were removed from the blood. These results suggest that the ketone acids removed by the muscle were oxidized to produce the high respiratory quotients, while the low respiratory quotients resulted from the incomplete combustion of fatty acids. If glucose is not oxidized in pan-

creatic diabetes, it is possible to ascribe the high respiratory quotients, in part, and a ketolytic effect, in its entirety, to the oxidation of lactic acid.

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CAPACITY OF SKELETAL MUSCLE IN RATS TO MAINTAIN WORK OUTPUT

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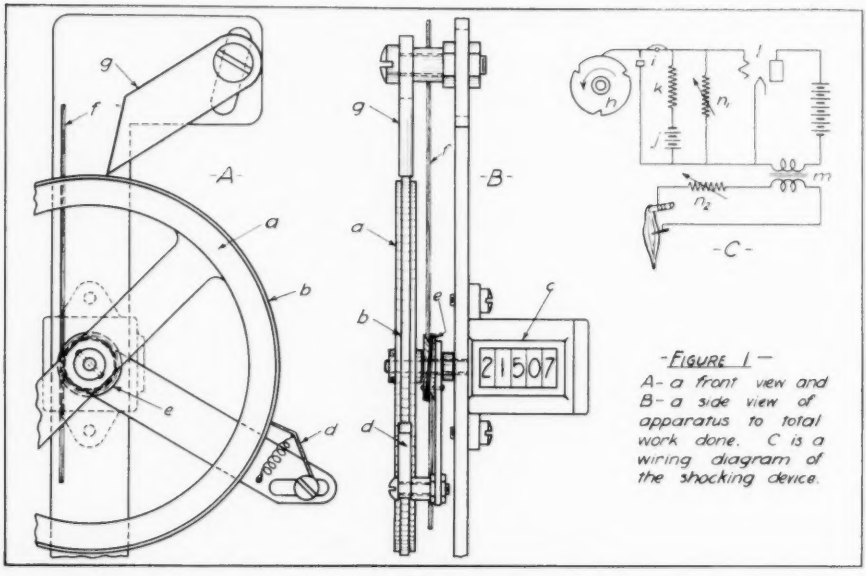
Studies on the work performance of skeletal muscle have invariably shown progressively increasing fatigue to be an outcome of rapidly repeated muscular contractions. This has been equally true for investigations of both isolated muscle and that of the intact animal. We are in a position to call attention to an interesting exception to the above generalization. It does not seem to have been anticipated by others. Gans and Miley (2) stimulated the gastrocnemius muscle of anesthetized rats through the sciatic nerve at the rate of once per second. They report 26 hours as the maximum time for complete fatigue. In no other study with which we are familiar has it been reported that contractions at a similar rate can be maintained for a longer time in skeletal muscle. Using modified methods we have observed contractions up to 17 days during which the muscle (gastrocnemius) lifted 100 grams 3 times per second. Under the conditions of our experiment we have never produced complete fatigue in the normal animal.

Apparatus. In figure 1, A and B show front and side views respectively of the apparatus used to total the work done by the animals. A very light aluminum wheel, *a*, is attached to the shaft of a Veeder counter, *c*. A rubber band is attached to the periphery of this wheel. Upon this rubber band rests a light dog, *d*. This dog is attached to a lever which pivots freely on the shaft of the counter. Fixed to the shaft end of this lever is a small pulley, *e*, around which a cord, *f* is passed. The upper end of this cord is attached to the muscle of the rat and the lower end to a 100 gram weight. Therefore, when the muscle contracts, the dog, *d*, is pulled downward and the weight is pulled up. When the muscle is relaxed the weight pulls the dog back to its original position and at the same time turns the wheel, *a*, which in its turn operates the counter, *c*. The stationary dog, *g*, prevents the wheel from moving in the wrong direction when dog *d* is pulled downward.

Figure 1 C shows diagrammatically the wiring of the shocking apparatus. A notched wheel, *h*, is attached to the shaft of a small telechron motor which runs at a speed of one revolution per second. This caused a con-

tact to be made and broken at *i*. When the contact is made, the 45-volt battery, *j*, is shorted through the fixed resistor, *k*, of 10 megohms. This removes the grid potential of the tube, *l*, and current flows in the plate circuit through the primary of the small audio transformer, *m*, which has a ratio of $3\frac{1}{2}$ to 1. The diagram shows a battery as the source of the plate current. However, actually a "B-eliminator" was used with a rated output of 180 volts.



-FIGURE 1-
A- a front view and B- a side view of apparatus to total work done. C is a wiring diagram of the shocking device.

Fig. 1

Fig. 2. Oscillographic record of the shock used. The three shocks shown are each from a different notch on the revolving wheel.

The use of this "B-eliminator" accounts for the fact that there was a slight amount of alternating current present in the output of the device as shown in the figure 2. This photograph was made with a Westinghouse oscillograph. The general form of the shock used may be seen from this record. The AC component was not sufficient to maintain a state of tetany

in muscle except for the first few minutes of the work period. Whether it had any other effect, we have been unable to determine.

The intensity of the shock may be changed by either variable resistor (n_1) or (n_2).

METHODS. The animal is anesthetized with sodium phenobarbital (Merck). The distal end of the tibia is exposed and secured in a vertical position by a hemostat. A silver needle is inserted between the gastrocnemius and biceps muscles exactly on a horizontal level with the knee-joint. The hemostat and needle serve as electrodes. A 100 gram weight is attached by strong linen thread to the freed tendon of the gastrocnemius. The thread is looped around the pulley of the recorder.

TABLE 1

Ten-day work records expressed in kilogram-meters for each 24 hour period

SEX	WEIGHT*		DIET†	1	2	3	4	5	6	7	8	9	10	TOTAL
	A	B												
m	233	213	B	197	118	132	142	133	132	132	134	132	132	1,384
m	270	207	B	231	190	193	187	166	152	146	142	138	147	1,692
m	364	345	C	160	182	177	180	178	158	164	161	184	178	1,722
m	312	272	C	193	163	110	100	81	70	76	72	72	79	1,016
f	197	123	A	67	56	47	40	31	33	39	40	30	33	416
m	360	220	A	184	153	153	156	159	150	142	134	116	129	1,476
m	279	157	A	185	158	166	149	123	99	93	86	69	58	1,186
m	310	275	D	183	176	187	147	146	163	165	161	158	156	1,642
m	305	200	C	165	166	196	217	213	204	212	204	182	206	1,965
m	296	194	C	202	264	221	228	206	183	209	218	233	219	2,183

* Weight: A is weight in grams at beginning of work period. B is weight in grams at end of work period.

† Diet: A, a 10 per cent dextrose solution; 1 cc. per 50 grams body weight every 8 hours; B, 10 cc. condensed milk daily in addition to diet A; C, 7 grams dextrose, plus 7 grams mash and 15 cc. water by mouth daily; D, 20 grams condensed milk plus 10 grams dextrose by mouth daily.

Food may be administered orally during the work period. We have not yet standardized a diet suitable for 10-day periods. In experimental problems requiring only a five day work period the administration of a 10 per cent dextrose solution by the subcutaneous route (1 cc. per each 50 body weight every 8 hours) is entirely satisfactory. Most adult animals will work for five days and longer when only water is administered.

Records are taken every eight hours. At this time the animal receives food and appropriate amounts of anesthetic. Washing the lower leg with a tincture of metaphin at these intervals is completely effective in preventing infection.

RESULTS. Sample work records over a 10-day period are summarized in

table 1. Performance among animals is variable. It is significant to note several instances in which energy output for 24 hours exceeds that for previous days. Although the height of initial contractions may diminish by 30 per cent to 40 per cent during the first 15 minutes of work we have on several occasions seen the initial contractions exceeded in height several days later.

It is usually necessary to suspend experimentation by 10 days because of a sloughing of the muscle. Considerable amounts of the muscle are inactivated in this way and an inevitable outcome is the breaking of the tendon from the muscle. This has been delayed to a maximum of 17 days in one case. At the end of this period the height of muscular contraction was still 30 per cent that of initial contractions.

Discussion. Others working along similar lines have failed to find work performance of the order reported here. For the work of Gans and Hoskins (1), Gans and Miley (2), and of Miley (5) we can point out a limiting factor in methods employed by them. In preliminary experimentation we followed their plan of stimulating the muscle through the severed sciatic. Times for "fatigue" ranged from 18 to 35 hours. It was possible to show beyond a doubt that this decrease in irritability was a consequence of the dying of the nerve and not dependent to any important extent upon work itself. We were led away from the latter conclusion through observing the accompanying loss of irritability in the muscle itself. This loss of muscular irritability is a consequence of denervation as can be demonstrated by severing the sciatic and attempting stimulation of the muscle after a 36 hour delay. The muscle may or may not respond maximally upon direct stimulation and stimulation through the nerve elicits no response. After stimulation of from 5 to 30 minutes muscular contractions will be completely abolished. Control operations showed that the procedure of exposing the nerve was in no way responsible for decreased irritability. Possible changes in circulation and water balance of the muscle tissue may be responsible. This is only a suggestion.

In the investigations by Hartman, Brownell, and Lockwood (3) and by Leese, Hines, and Jorden (4), relatively short periods of work were reported although the method of direct stimulation was used. In use of several different set-ups for stimulation of muscle we have found that amount of work done varies markedly with type and strength of stimulus applied. In none of our experiments, however, have we observed "fatigue" in so short periods as were reported by them.

The use of a supramaximal shock allows a decrease in muscle irritability without decrease in height of muscular contraction. When it is recalled that a rat may run 15 miles or more per day through voluntary effort the performance of one muscle in an otherwise resting animal does not seem so remarkable.

It is probable that several factors in addition to work itself contribute to the decrease in height of contraction occurring after several days of work. The marked loss of body weight which we were usually unable to prevent by feeding might well be expected to weaken the animal. At autopsy all traces of body fat are usually absent. Muscles other than the working muscle appear to be atropic. Abnormal retention of food in the stomach and intestine tends to mask the true weight loss. Scabbing and sloughing around the freed end of the muscle cause friction and in the later parts of a ten-day interval have reduced the amount of active muscle considerably. For these reasons we have found it advisable in applications of our methods to limit the work period to five days.

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RECIPROCAL CHANGES IN REFLEX ACTIVITY OF THE FORE LIMBS INDUCED BY POST-BRACHIAL "COLD-BLOCK" OF THE SPINAL CORD[1]

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A century ago Marshall Hall (16) published his well known account of the augmentation ("release") of spinal reflexes which follows destruction of the frog's brain. Somewhat later (1850) he described the brief depression ("shock") of limb reflexes produced by transection of the spinal cord, also of the frog (17). It is an early, though less familiar observation, that the changes in reflex excitability produced by a spinal transection are *not* confined to the spinal cord segments *below* the level of injury. Schiff (37) in 1858 observed in normal and decerebrate frogs that an increased irritability of fore-limb reflexes was induced by severing the spinal cord below the brachial enlargement. He believed that the hyperreflexia so produced was not due to irritation of ascending spinal tracts because it was still evident a month after the operation.

Sherrington (39) in 1898, without knowledge of Schiff's experiments, described in greater detail a similar phenomenon in the mammal. Therefore, whenever an eponym is desirable, cephalad release of function should probably be designated the Schiff-Sherrington phenomenon. Sherrington observed that reflexes elicited from the skin of the head were augmented by a transection of the spinal cord in the upper cervical region. Moreover, a post-brachial transection of the spinal cord subsequent to an initial cervical transection increased the reflex irritability of the fore limbs. The crossed extensor reflex, though unobtainable in the fore limbs after the cervical transection, was easily elicited after the second more caudal

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section. No sign of spinal shock or depression of reflexes above the level of section was noted.

Fulton, Liddell and Rioch (13) discovered an analogous phenomenon within the lumbar enlargement. In their experiments, an initial transection or hemisection in the thoracic region was followed after a short interval by a secondary transection immediately below the spinal roots which innervate the quadriceps extensor muscle. The depression of extensor reflexes induced by the initial thoracic section was considerably mitigated by removal of the influence of the segments below L 5 or L 6. Brief accounts of the augmentation of fore-limb extensor reflexes by post-brachial transection in the decerebrate cat have been given by Denny-Brown and Liddell (6), Liddell, Matthes, Oldberg and Ruch (21), Ruch (34), Ruch and Watts (35) and Miller (26).

The instances of the Schiff-Sherrington phenomenon noted above have been largely incidental observations. Nevertheless, they suggest an important exception to cephalo-caudal polarity in the functional organization of the central nervous system. They suggest further that the excitability of segmental reflexes depends upon remote regions of the spinal cord as well as upon brain stem nuclei.

METHODS. The effect of post-brachial transection of the spinal cord upon flexor and extensor reflexes of the fore limbs has been examined with Sherrington's technique (12) for optical isometric recording of reflex contractions in isolated muscles, elicited by mechanically controlled stimulation. The fact that such experiments can be performed only upon acute preparations (decapitate or decerebrate) necessitates the use of acute surgical transections, which may produce traumatic artifacts. To avoid these difficulties, surgical procedures have been supplemented by narcotization of the severed ends of the spinal cord or supplanted by a "cold-block" or "novocaine-block" of conduction in the spinal cord.

All experiments were performed upon cats decerebrated at an intercollicular level by the trephine method. The muscles selected for recording were the brachialis anticus muscle and the lateral together with the medial (short) head of the triceps muscle, which unlike many brachial muscles (8) are relatively pure physiological flexors and extensors, respectively. One of this antagonistic pair of muscles was dissected free at its distal end from surrounding soft tissues without injury to blood or nerve supply. The muscle was isolated further from direct and from reflex effects of contraction in adjacent muscles by severance of all other nerves supplying the brachium and antibrachium except the motor nerve to the recording muscle.

The steps taken to ensure the isometric conditions necessary for accurate quantitative measurement of the strength of reflex contraction were: 1, immobilization of both heads of the humerus by twist drills caught in rigid clamps screwed to a heavy experimental table; 2, direct attachment of the muscle to the myograph lever by a light steel hook without intermediation of strings or pulleys; 3, the use of a stiff torsion-strip and optical magnification of its movements; 4, rigid support of the myograph by stressed steel girders built into the framework of the building. The

myograph consisted of a spring steel torsion-strip secured at both ends and therefore free from external friction.

The index of extensor activity employed was the reflex response to a stretch applied by the "fall-table" device of Liddell and Sherrington (22, 23). The heavy top of the "fall-table" to which the humerus was securely clamped was arranged to descend of its own weight against the resistance of a valved oil plunger. The muscle, being the only bridge between the unyielding myograph and the heavy table top, was subjected during the descent of the table to a slowly and evenly applied stretch of controlled magnitude and speed of application. Reflex contraction in the flexor muscle was elicited by stimulation of a bared ipsilateral nerve through Sherrington glass-shielded electrodes with single and repeated induction shocks.

In an effort to avoid trauma, irritation and deterioration of the preparation, which may result from surgical transection of the spinal cord in the unanesthetized animal, we employed three special methods of interrupting conduction in the spinal cord, namely: 1, surgical transection combined with narcotization of the rostral cut face of the spinal cord; 2, novocaine-block; and 3, localized cooling or cold-block of the spinal cord. Freezing has long been used in place of surgical procedures for blocking peripheral nerves (Gad, 14) or the spinal cord (Pike, 29). In 1910 Trendelenburg described a method of *reversible* cold-block for producing a transient and non-irritative interruption of function in the central nervous system, which he used with success upon the medulla oblongata (44), the motor region of the cerebral cortex (46), and the spinal cord (45). Trendelenburg (45), Sherrington (41), and Beaman and Davis (1) produced with slightly different methods of localized cooling the essential changes in reflex excitability which follow surgical transection of the spinal cord at the same level, but without any sign of the disturbing stimulation which accompanies the latter.

The method of procuring the cold-block which we have found most satisfactory is similar to that of Trendelenburg and of Beaman and Davis. The laminae of at least three thoracic vertebrae were resected and the middle lamina was notched deeply to the floor of the vertebral canal. A rubber tube 2 to 4 mm. in diameter, made of readily obtainable fine rubber dam (Latex), was then passed around the spinal cord outside the dura mater. After mounting the preparation in the myograph, one end of this tube was connected through a Y-tube with two reservoirs. One contained water cooled to 4°C. by a coil carrying refrigerated brine; the other contained water at 37 to 40°C. The procedure in our experiments consisted of recording one or more reflex contractions during alternate periods (usually 5–10 minutes) in which warm or cold water was passed through the rubber tube in intimate contact with the spinal cord.

RESULTS. *The stretch reflex of the triceps muscle.* The stretch reflex, originally described by Liddell and Sherrington (22, 23) is the reflex contraction of an antigravity muscle to an afferent influx originating within the reacting muscle itself when lengthened or stretched; it is the unit reflex of posture. The stretch reflex is fundamentally a spinal process in the cat (Matthes and Ruch, 25) and in the dog (Denny-Brown and Liddell, 7). But it is highly dependent upon facilitation from supra-spinal nuclei since it ranges from complete inexcitability in the acute spinal animal to exaggerated excitability in the decerebrate preparation. This feature of the stretch reflex makes it a sensitive index of any change in the

facilitative or inhibitory background produced by a post-brachial transection of the spinal cord.

The effect upon the fore-limb stretch reflex of a post-brachial cold-block. The effect of a post-brachial cold-block of the spinal cord in our experiments has been uniformly to increase the magnitude of the reflex response of the triceps muscle to stretch. Often this increase has been great; in no experiment has the reverse change, a decrease in the reflex tension developed, been observed.

Our results on this point are presented in the form of tracings from original records of the stretch reflex obtained during the passage of cold and warm water through the tube encircling the spinal cord in the thoracic region. The records of a series were gained within a short space of time and with unaltered initial tension and stretch stimulus. The tracings are labelled to indicate whether they were made during a period of cold-block *C*, or after rewarming the spinal cord, *W*. Records *E* were made with the tube collapsed and the spinal cord at normal body temperature. The figures attached to each letter give the order in which the records were obtained. *T* is the signal which records the application of the stretch stimulus and serves to divide the record into an initial static period, a phasic period and the static period. The P-curve represents the tension produced by the muscle after severance of its motor nerve (peripheral component), but in all other respects the muscle was acting under the same conditions that obtained for the reflex records. Subtraction of the P-curve from the total response gives the active reflex component.

Figure 1 shows the alteration in the excitability of the triceps muscle produced by a post-brachial cold-block, which was instituted and remitted four times in the same experiment. The reflexes recorded during the cold-block ranged in the four series between 142 and 281 per cent of the normal, *W*, during the initial static period and between 128 and 152 per cent during the static period. In figure 2 from a similar experiment the effect of the cold-block can be estimated more accurately since the reflex response can be dealt with separately from the peripheral component of the total response. These records illustrate clearly the magnitude of the increase in reflex activity which may be produced. In series A of figure 2, recorded early in the experimental session, the pure reflex response to the same initial stretch was rendered six times greater; the pure phasic stretch reflex was approximately doubled.

In order to compare cooling and surgical transection of the spinal cord, some experiments (fig. 1) were terminated by a surgical transection closely adjacent to the cooling tube after removal of the previous cold-block. The percentage increase in the magnitude of the stretch reflex was close to that produced earlier in the experiment by cooling the spinal cord in the same region. This result was typical of four experiments.

which indicates that a complete functional "transection" of the spinal cord is produced by short periods of cooling.

In other experiments a procedure was adopted to control the possibility that cooling the spinal cord stimulates ascending spinal tracts or that cold applied to the thoracic region spreads to the brachial enlargement. The spinal cord was severed surgically one segment below the cooling tube. Novocaine was applied to the rostral cut face of the spinal cord and a short

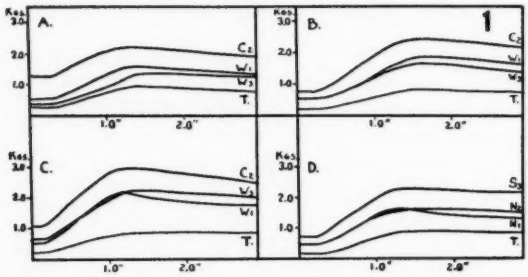

Fig. 1. The stretch reflex of *m. triceps* after cooling and section of the spinal cord in the thoracic region. A, B, and C are the first, third and fourth series in same experiment. Tracings labelled *C* were made with cold water passing through a tube around the spinal cord; for *W*-tracings warm water was flowing, and they constitute normal decerebrate or control reflexes. *T* is the signal for the stretch stimulus. The small index figures give the order of the observations. D, surgical section; *N*, before section; *S*, after section. The preparation was in the prone position. Ordinates are kilograms isometric tension.

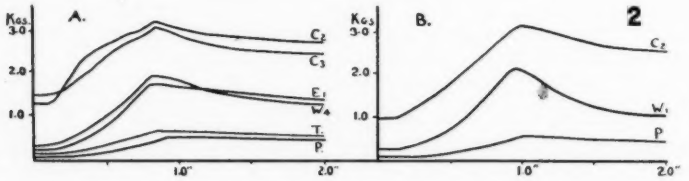

Fig. 2. Same as figure 1. The *P*-curve is response of the muscle to stretch after section of its motor nerve; *E*-curve, tube empty and spinal cord warm. *C* or *W* minus *P* is the active reflex component.

period of time was allowed for subsidence of irritation and facilitation from the transection. Cold and warm water were then passed through the cooling tube alternately. In three such experiments the myograms of the stretch reflex recorded during cold and warm period were identical, although before transection the cooling had been clearly effective. This result is in agreement with those of Trendelenburg (45), who showed by thermocouple measurements that cooling the spinal cord of the rabbit

(said to have no anterior spinal artery) in the thoracic region produced no fall in the inter. al temperature of the lumbar enlargement.

Post-brachial novocaine-block. The result obtained with the cold-block technique has been reproduced with another method of functional "transection," a novocaine-block. A deep laminectomy was performed in the thoracic region and the dura mater opened and reflected by four hemostats. The pia-arachnoid was nicked in several places and thin cotton pledgets saturated with 2 per cent solution of novocaine were applied to the spinal cord around its whole circumference. The results of such an experiment are shown in the form of a "protocol-graph" in figure 3. Within five minutes of the initial application of novocaine the reactivity of the stretch reflex was somewhat increased and within 15 minutes the full increase was reached. Irrigation of the narcotized area of the spinal cord with warm

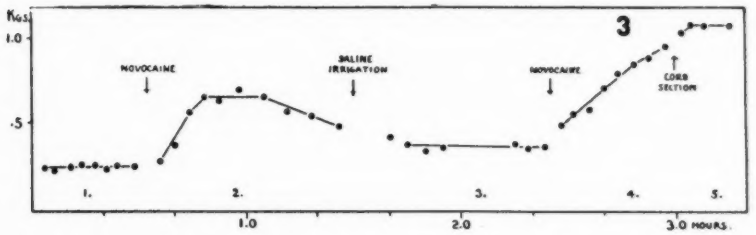

Fig. 3. Protocol-graph showing effect of a post-brachial novocaine-block on the stretch reflex of the fore limb. The successive segments of the curve are 1, control decerebrate reflexes; 2, partial novocaine-block; 3, second control series after saline irrigation; 4, second novocaine-block; 5, after surgical transection within the novocaine block. Ordinates: kilograms of pure reflex tension, i.e., after subtraction of the peripheral component.

saline for twenty minutes was sufficient to dissipate the narcotic block. During the second, as during the first application of novocaine, the active reflex component of the stretch reflex was increased one and one-half times. A surgical transection at the same level produced no greater increase in reflex excitability than could be reasonably expected from a slightly more prolonged narcotization. A complete spinal block can apparently be obtained with facility by application of novocaine to the surface of the spinal cord. Figure 3 illustrates a virtue of functional transection over surgical transection in addition to the control of irritation afforded. Rarely can so great a uniformity of successive records be obtained from a preparation subjected to the violence of surgical transection without anesthesia. It is felt that the use of a novocaine block greatly increases the nicety and reliability of an acute experiment demanding transection of the spinal cord in unanesthetized preparations.

The fore-limb flexion reflex after post-brachial transection. Previous investigations of the effect of thoracic transection of the spinal cord upon fore-limb reflexes have been concerned with reflexes of extension alone, or have not distinguished clearly between reflexes of extension and flexion. A series of experiments similar in plan to the preceding ones on the triceps muscle has been carried out upon the flexion reflex of the fore limb. These experiments were prompted by the belief that limited and sometimes erroneous interpretations are made when the results of lesions of the nervous system are studied in only one type of reflex, either flexion or extension.

A protocol-graph showing the effect of post-brachial cooling of the spinal cord upon the flexion reflex of brachialis anticus muscle is shown in figure 4. In this experiment the flexion reflexes elicited after a brief passage of cold water through the tube around the spinal cord were of much less magnitude than the responses to a stimulus of the same strength before cooling. Rewarming the cord caused a return of the reflex to normal. The maximum reduction in tension observed in this experiment was 1.4 kgm. The responses elicited during the last four cold periods were 48, 72, 34 and 54 per cent, respectively, of the control responses to the same stimulus in the immediately preceding warm period. Figure 5 shows the values for the first three cold periods plotted according to their position within their respective cold periods to show the rapidity with which the effects of cooling are manifested. The decrease in flexor excitability in these instances started in the second minute and was well advanced by the end of the third minute. This experiment confirms the impression gained from clinical observations of a relatively sudden onset of the cold-block.

The depression of flexion reflex excitability was not usually so pronounced as that seen in figure 4. The usual result was a reduction in reflex tension during the cold periods to 60 or 80 per cent of the normal (cord warm). Thus in an experiment in which the cold-block was instituted and remitted three times, the reflex tension was reduced in successive periods to 67, 81, and 79 per cent of the control values. The decreased vigor of the flexion reflex of the fore limbs was always accompanied by a disappearance of decerebrate rigidity in the hind limbs and an obvious increase in the excitability of the hind-limb flexion reflex. In no instance was the flexion reflex of the fore limb increased by post-brachial cooling of the spinal cord.

It has been possible to produce marked depression of fore-limb flexion by post-brachial surgical transection, though not with the same uniformity. Figure 6 shows an experiment in which the normal reflexes were more than twice as large before than after transection of the spinal cord. For the reasons given under *Method* we are inclined to place less confidence in the results of surgical than in the results of functional "transection" of the spinal cord of unanesthetized preparations. Besides the facilitation and deterioration produced by transection, the storm of activity produced by

surgical transection makes difficult the exact duplication of the conditions of stimulation (electrode placement, etc.) before and after the transection.

Reversibility of the cold-block. The blocking of conduction produced by localized cooling of the spinal cord was not in all experiments immediately reversed by rewarming the spinal cord. In figure 7 is shown an experiment

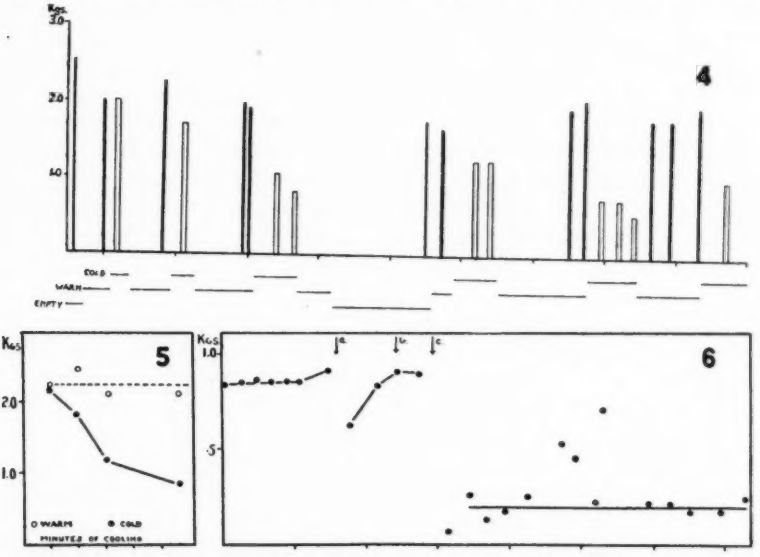

Fig. 4. Protocol-graph showing the effect upon the ipsilateral flexion reflex of the fore limb of alternate warming and cooling the spinal cord in the post-brachial region. Afferent nerve: superficial radial. Muscle: *m. brachialis anticus.* Tetanic stimulation. Solid lines (controls) are records made while spinal cord was warm; unfilled blocks are responses during cooling; horizontal lines at bottom show the duration of warm and cold periods. Time: 10 minute intervals. See also figure 5.

Fig. 5. The values from the first three cold periods of figure 4 plotted according to position of each in its own cold period.

Fig. 6. Single-shock, ipsilateral flexion reflex of fore limb after thoracic spinal transection. Muscle: brachialis anticus. Afferent nerve: ulnar. Time: 20 minute intervals. At arrow *a* the wound was opened, at *b* novocaine swabs were applied to the cord, at *c* the cord was divided at level of Th. 10 and novocaine was applied to the rostral cut face of the spinal cord. After transection, the hind-limb flexion reflex was unusually active, the extensor reflexes almost inelicitable. There was "walking" in both fore and hind limbs.

in which cooling the spinal cord for a period as short as 5 minutes produced a depression of the flexor reflex which apparently persisted for 30 minutes and which rendered ineffective a subsequent application of cold and a surgical transection. In other experiments the first application of cold allowed a return to normal reflex excitability, but later applications were

followed by slower and less complete return to normal excitability. No satisfactory explanation of the irreversible effects of cold upon the spinal cord was discovered. It was manifested simultaneously in both flexor and extensor reflexes, and in both fore and hind limbs. It is probably not due to a "pressure-block" of the spinal cord because replacement of warm by cold water involved no change in the diameter of the cooling tube; because irreversible blocks occurred when a rigid tube was employed for cooling; and because no similar effect occurred as the result of collapsing and refilling the tube with warm water. The irreversible change seems clearly to date from the period of cooling and must therefore be an effect of low temperature directly or indirectly upon the fibers of the spinal cord. It is suggested that cooling may by intense vasoconstriction produce anemia or some other local change which outlasts the period during which the temperature of the spinal cord is below normal.

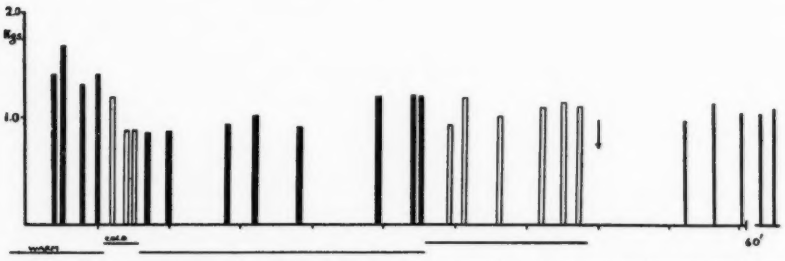

Fig. 7. Same as figure 4. Time: 10 minute intervals. A copper tube was used for cooling. Note the absence of recovery after the first cold-block. The increase in the responses during the latter part of the second warm period is probably due to a change in the general excitability of the preparation since further cooling and transection were almost without effect. The arrow indicates spinal transection at the level of the cold-block.

Discussion. Interruption of the spinal cord below the brachial enlargement, which spares the descending tracts from the brain to the brachial enlargement, greatly augments the excitability of extensor reflexes and diminishes the excitability of flexor reflexes of the fore limbs. These alterations in the reflex excitability of brachial muscles are probably not due to irritation of ascending tracts, or to a prolonged excitement of the preparation, because the changes occur after functional interruption of spinal conduction by localized cooling or by narcotization of the spinal cord. The same changes also occur after post-brachial surgical transection combined with narcotization of the rostral end of the severed spinal cord. Since the main blood supply of the cervical spinal segments is not interfered with, little attention need be paid to vascular factors, which are often invoked to account for reflex changes associated with spinal transection.

The hyperreflexia of extensor reflexes constitutes a special case of release of function—a release phenomenon rostral to the transection. According to current concepts of spinal cord function, this hyperreflexia suggests the existence in the intact animal of ascending tracts which, directly or indirectly, exert a continuous restraining or inhibitory action upon the extensor motor neurones of the fore limbs. Similarly, the concomitant depression of the flexion reflex implies the interruption of an ascending stream of impulses which facilitates the flexion response. Thus, contrary to the usual statement, *a depression of certain reflexes (akin to spinal shock) takes effect rostral to a spinal transection.*

The Schiff-Sherrington phenomenon constitutes a departure from cephalo-caudad polarity in the functional organization of the nervous system (dominance of the head segments, encephalization, etc.). In addition to the control of the fore limbs through tracts descending from the brain, there is also a strong control exerted from more caudal segments. The magnitude of this latter influence should be emphasized. The present experiments show that removal of it by a post-brachial spinal-block may double or treble certain phases of the fore-limb stretch reflex and reduce by half the tension of the flexion reflex. Ascending intraspinal facilitation and inhibition are therefore quantitatively of considerable importance relative to the more familiar action of descending tracts.

The phrase "neural balance" has been used by Graham Brown (3) to denote the relative excitability of flexion and extension reflexes of the limbs existing after lesions of the nervous system. That the neural balance of the divided spinal cord favors flexion has been generously confirmed by the systematic studies of Sherrington (39, 40), Graham Brown (4), and Head and Riddoch (18, 33), and many others (9, 36) upon the "spinal" cat, monkey and man. It is often tacitly assumed that the neural balance of the isolated spinal cord represents a fundamental balance in the central disposition of the segmental afferent influx. The extensor pathway is held to be less complete within the spinal cord, i.e., more encephalized, than the flexion reflex pathway. While this is probably true in a measure, the results of our experiments, particularly when taken together with those of Sherrington (39) and Fulton, Liddell and Rioch (13) suggest that the neural balance of the isolated spinal cord is partly the resultant of excitatory and inhibitory influences from more caudal segments. The ascending impulses have been shown to be facilitatory to flexion and inhibitory to extension and, therefore, would favor the balance between these which is found in the isolated spinal cord.

The third aspect of the behavior of the fore limbs to post-brachial transection which should be emphasized is that antagonistic flexor and extensor reflexes are affected oppositely or reciprocally. This feature is also characteristic of the reflex changes below a spinal transection provided only

few of the main descending systems are involved. When the spinal cord of a preparation exhibiting decerebrate extensor hyperreflexia is transected, the extensor reflexes become much less irritable and some become wholly inelicitable. At the same time, as the experiments of Sherrington and Sowton (43), Graham Brown (4) and others (9, 10, 11, 21) have shown, the excitability of the flexor reflex is greatly augmented. The threshold for the single-shock flexion reflex decreases on the average tenfold (21). Thus when the spinal cord of the decerebrate preparation is transected in the thoracic region the flexion reflex of the lower limb and the extension reflex of the upper limb become more active. The flexion reflex of the fore limb and the extension reflex of the hind limb become less active. In both fore

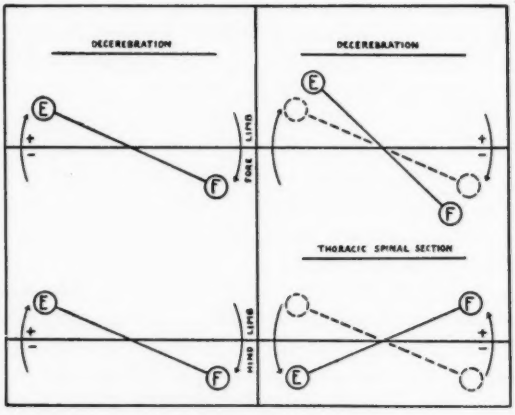

Fig. 8. A schematic diagram of the effect of thoracic transection of the spinal cord upon the reflex activity of fore and hind limbs of the decerebrate preparation. *E* and *F* are the extensor and flexor "half-centers." The ordinates are reflex excitability and indicate decrease and increase and not absolute amount of change in reflex excitability.

and hind limbs, therefore, the excitability of flexion and extension reflexes undergoes a reciprocal alteration. Furthermore, the same "type-reflex" is affected oppositely in fore and hind limbs. These changes are set forth schematically in figure 8.

Such reciprocal effects of transection produce what has been described by Graham Brown (3) as a "tilting of the neural balance." In the view of the writers, the functional organization of the nervous system which underlies neural balance is a reciprocal action of the long tracts of the spinal cord upon the final common path, in which one "half-center" is facilitated and the other is inhibited. Reciprocal innervation appears to characterize the activity of descending tracts excited by natural stimuli as in tonic neck and labyrinthine reflexes (Beritoff, 2) and by electrical stimulation of the

motor cortex (Sherrington and Hering, 42). If the tonic or continuous activity of descending tracts is similarly reciprocal, then interruption of them would produce reciprocal changes in reflex excitability such as our experiments and those of others have demonstrated. The intimate details of the neural mechanism for reciprocal innervation are of course as obscure for the descending tracts as for spinal reflex arcs. Perhaps the most economical view is that descending tracts employ or act through the spinal mechanism for reciprocal innervation (12). Recent unpublished experiments of McCouch (24) support this suggestion. Such an arrangement is easily conceived for those descending fibers which terminate upon the internuncial neurones of the segmental reflex arcs (Rasdolsky, 32; Hoff, 19).

Inhibition of spinal reflex activity by the brain has from the first (Setschenow, 38) too often been treated as an independent agent or faculty, entirely divorced from active reflex postures mediated through brain-stem nuclei. Purely inhibitory nuclei and tracts have been freely described on the basis of observations confined to but one type of reflex. This unphysiological view of inhibition has often been attacked and alternative hypotheses which explain post-transection hyperreflexia without resource to release from inhibition have been advanced. Such are the attempts to ascribe release phenomena to anoxemia of the spinal cord (Walshe, 47); to a reaction to degenerating nerve fibres (Langley, 20); to a post-transection evolution of reflex connections (Rudolf, 36, and Minkowski, 27) or to a compensatory increased activity of previously existing ones (Pike, 30); to a concentration of "nervous-energy," normally irradiated, into spinal reflex paths (Schiff, 37; Munk, 28). Furthermore, the literature yields the impression that "shock" and release of function are sometimes considered to be mutually exclusive phenomena. Spinal shock has been interpreted as an inhibitory phenomenon (Goltz, 15) and release phenomena have been held to be an overswing of the process underlying the recovery from spinal shock as in the "isolation alteration" theory of Munk (28). None of these theories accounts adequately for the suddenness of onset, the permanence, and the reciprocal character of the reflex changes induced by spinal transection.

The hypothesis that descending and ascending tracts of the spinal cord exhibit reciprocal innervation suggests that exaltation of function (spinal release) and depression of function (including spinal shock), far from being incompatible events, are on the contrary probably never separate occurrences. They appear to be two aspects of the same event, the cessation of streams of impulses with a reciprocal distribution over spinal motoneurones. Whenever a single homogeneous descending system is interrupted, "release" predominates in one "half-center" and depression predominates in the other, and the result is a shift in the neural balance. Therefore in this view, reciprocal changes between flexor and extensor

reflex excitability, neural balance, release of function and spinal shock can be traced to a single feature of the functional organization of the nervous system—reciprocal action of ascending and descending spinal tracts *upon* spinal motoneurones or *through* segmental reflex arcs.

SUMMARY AND CONCLUSIONS

In an investigation of the cephalad effects of spinal transection, the ipsilateral reflex of a flexor muscle and the stretch reflex of an extensor muscle of the fore limbs have been recorded from "isolated" muscles by an optical isometric myograph after surgical transection and after functional block of the spinal cord by cooling and by novocaine.

1. The excitability of the stretch reflex of an extensor muscle of the fore limbs is *augmented* by blocking spinal conduction below the brachial enlargement.

2. The excitability of the ipsilateral reflex of the antagonistic flexor muscle is *decreased* by the same procedure.

3. These changes are due to interruption and not irritation of ascending tracts, because they are produced by functional block of the spinal cord by cooling or narcotization and by surgical transection combined with narcotization of the rostral cut end of the spinal cord. They are, therefore, interpreted as cephalad "release" and depression phenomenon respectively, such as occur below a spinal transection.

4. The complete syndrome of mid-thoracic transection of the spinal cord in the decerebrate cat includes reciprocal changes in reflex activity between flexor and extensor muscles of the fore limbs, between flexor and extensors of the hind limbs, and between flexors of the fore and hind limbs, and between extensors of the upper and lower limbs.

5. The basis of the neural balance is discussed.

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FACTORS DETERMINING BLOCK OF THE CONDUCTED CARDIAC IMPULSE

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The phenomenon of irreciprocal conduction or monodromia (Schmitt and Erlanger, 1928) is well known to experimenters in the field of cardiac impulse conduction and block. The phenomenon may be observed in skeletal muscle (Engelmann, 1896), in hearts (Engelmann, 1895; Mines, 1914; Skramlik, 1920) and heart strips (Erlanger, 1906; Schmitt and Erlanger, 1928; Ashman and Hafkesbring, 1930). Completely unidirectional conduction (monodromia) or conduction better in one direction than in the other (heterodromia) frequently occurs in preparations in which different environmental conditions exist on the two sides of a natural junction or of an artificially produced region of depression. Engelmann and those who have followed him, however, were often unable to predict the direction in which the unidirectional conduction would occur. Engelmann's experiments upon the sartorius muscle of frogs (1896) were apparently consistent enough to allow reasonable foundation for his statement that conduction occurs better in the direction of faster reacting to slower reacting tissue than in the opposite direction. Engelmann's experiments upon the heart seemed to have yielded less predictable results. This unpredictability of directional effects of asymmetrical blocks applies in general to the experiments of later workers. Erlanger reports that monodromia was frequently seen in strips which had been conducting for some time in one direction and to which stimuli were then applied at the other end, this reversal frequently resulting in complete block. Ashman and Hafkesbring (1930) report experiments with an asymmetrically shaped clamp in which the results appear to have been generally consistent although they were not invariable.

We have examined first, the data of previous workers; secondly, data of our own obtained when junctional block occurred in intact hearts subjected to vagus inhibition, and, thirdly, data obtained from heart strip preparations subjected to pressure block. From such data, we have been led to the conclusion that the factors involved in the development of heart block

and heterodromias are such as to make improbable the invariable predictability of the direction of a monodromia.

The experiments presented below were performed in an attempt to demonstrate the importance of three factors in the determination of conduction or block at a region of interference. These three factors may be varied more or less independently and only by knowledge and consideration of all three can the occurrence or prevention of conduction through a blocking region be predicted.

METHODS. Strips 30 mm. to 35 mm. in length were cut from the ventricles of turtles, usually *Pseudemys elegans*. The strips were subjected to compression by means of a rubber cuff 1 cm. in width. This cuff consisted of a tube made from medium weight dental rubber dam and placed inside another short tube of hard rubber of 1 cm. inside bore. By inserting a length of the rubber dam tube within and tying the ends over those of the hard rubber tube support was given to the former. A hole drilled through the hard rubber wall and provided with a metal side tube allowed the injection of air or mercury into the space between the flexible rubber tube and the rigid wall. Connection of the side tube with a mercury levelling bulb allowed known pressures to be applied to the cuff. With the tubing used, 30 cm. mercury pressure could be applied safely to the system. This pressure was sufficient to produce a convenient degree of block of most strips in about half an hour and was the pressure ordinarily used. Such a preparation could usually be used for from one to three hours before the degree of block became too severe to allow of any conduction through the compressed region. An applied pressure of 40 cm. mercury was frequently tried. Such a pressure usually produced a high degree of block in a few minutes but the preparations so obtained were rarely stable enough to give properly controlled series of records. Furthermore, the 40 cm. mercury pressure was close to or above the pressure which could be sustained by the cuff. Consequently, when the levelling bulb was raised to give such pressures, the cuff frequently bellied out on one side and a prompt lowering of the bulb was necessary to save the cuff from stretching beyond its elastic limits and bursting. The injury produced in the heart strip by this asymmetric bulging of the cuff frequently produced monodromias and it was chiefly for this reason that the higher applied pressures were of any value. The experiments were performed during the winter at room temperatures of between 20°C. and 25°C.

Stimulation was accomplished by means of a Porter coil activated by a Thyratron device. The stimulator was arranged to operate by current from the 115 volt D.C. line but was otherwise essentially that described by Schmitt and Schmitt (1932). Two pairs of flexible wire stimulating electrodes were applied, one pair being at either end of the strip. A double-pole, double-throw switch connected the secondary of the induction coil

with either pair of electrodes as desired. By these means, rhythmic stimuli of any desired frequency could be applied to either end of the strip. Change of the end stimulated or of the interval between stimuli could be accomplished in less than a second.

Kymograph records were obtained by means of two heart levers attached to the two ends of the strip, respectively. A relay device recorded the moment of each stimulation.

CRITIQUE OF METHOD. Two possible complications arise in attempting to draw general conclusions from experiments done by the above procedure. One of these deals with the possibility of oversimplification of conditions in the heart strip preparations when these are compared with conditions at normal junctions in intact hearts. With respect to this possible objection, we would point out the conclusion formerly reached by Erlanger, namely, that since the phenomena of A-V conduction and block can be so closely reproduced in a strip preparation, it seems valid to argue from experiments on the latter with respect to the former. That histological differences exist between the two types of preparation we are well aware. It seems probable, however, that the underlying processes involved in the two cases are similar if not identical.

Another criticism which might be made of the method involves consideration of the extent of the depression caused by the compressing cuff. We have used the pneumatic cuff method because we have found such a cuff easier to control than a clamp of the Gaskell type. It is apparent that any form of mechanical or chemical injury produces effects which cannot be restricted to a perfectly limited region. However, it seems probable that, in a compressed strip such as we have used, the critical region of the strip may be considered as representing the localized region at which conduction from one side to the other is last maintained. The tissue on either side of this locus and immediately adjacent to it is almost certainly also depressed. The condition of the ends of the strip outside of the cuff is therefore of importance only remotely in determining whether an impulse is to be blocked or conducted.

THE FACTORS INVOLVED IN THE DETERMINATION OF BLOCK. The first factor in the determination of block will be referred to under the term "resistance." This includes the entire complex of depression produced by the cuff in the immediate region of the blocking locus. In the compressed strip, this complex is considered as equivalent to the complex in the intact heart present at a junctional region. No attempt is made here to determine, for the strip preparation, how much of this resistance factor is merely an expression of changes in the depressed tissue adjacent to the hypothetically acutely localized "junction." Greater pressure or prolonged use of the preparation increases the resistance factor. In the normal, intact heart with a good circulation, it is highly probable that the

resistance factor is essentially constant and is to be referred to a histological discontinuity in the conduction path. Changes in conduction time across such a junction in the intact heart are probably due chiefly, if not entirely, to changes in the adjacent tissue.

The second factor considered in the determination of block is that of relative refractoriness. It does not seem probable that the absolute refractory period is highly significant in determining the propagation of a physiological impulse across a junction. On the other hand, it seems of the utmost importance that great emphasis be placed on the course of the relatively refractory period which begins at the end of the absolutely refractory period and continues for some time thereafter. During this time the heart is recovering toward a condition of "normal irritability" from a condition in which it is totally unresponsive to electrical stimulation. It is probable that responsiveness to a physiological impulse follows a similar or identical course.

The third factor which we consider important in the determination of block will be referred to as the "exciting power" or "strength" of the conducted impulse from the proximal part of the strip or heart chamber. This term, like the term *resistance*, we prefer to leave without too strict a definition. If the action current alone causes restimulation ahead of an advancing wave of activity, this phase of our argument is in complete agreement with the discussion of Ashman and Hafkesbring (p. 79). The general thesis which we wish to present is thus essentially in accord with the hypothesis of Englemann and with the conclusions reached by Erlanger.

Results of experiments. The kymograph records reproduced as figures 1, 2 and 3 were selected from a large group which were obtained as demonstrating the basis of the conclusions which we have reached. The terms "upper half" and "lower half" which are used in discussing the records refer only to the levers to which the ends of the strip were attached. No significant differences were observed between ends of the strip from the right or left sides of the ventricle and no significance is given here to the position of the strip in the cuff. The white dots on the records indicate which end of the strip was stimulated by each shock.

In figure 1 are reproduced curves showing a situation in which the upper half of the strip was contracting with a 1:1 rhythm in response to impulses conducted from the electrically excited lower half. Following the conducted response A-A′, a premature contraction (B) of the upper segment in response to an applied shock caused failure of the upper segment to respond to the next physiologically propagated impulse from the lower half (C), even though the lower segment was stimulated after an interval longer than the previous intersystolic intervals. The experiment was repeated several times with slightly varying time intervals. Such a record is entirely analogous to that obtained from atrium and ventricle of an intact heart

when a ventricular extra-systole is followed by a compensatory pause due to blocking of the immediately following atrial impulse.

In the case of the extra systole (B) both the proximal and distal parts of the strip (upper and lower, respectively) were incompletely recovered from previous activity and a complete analysis is therefore impossible. How-

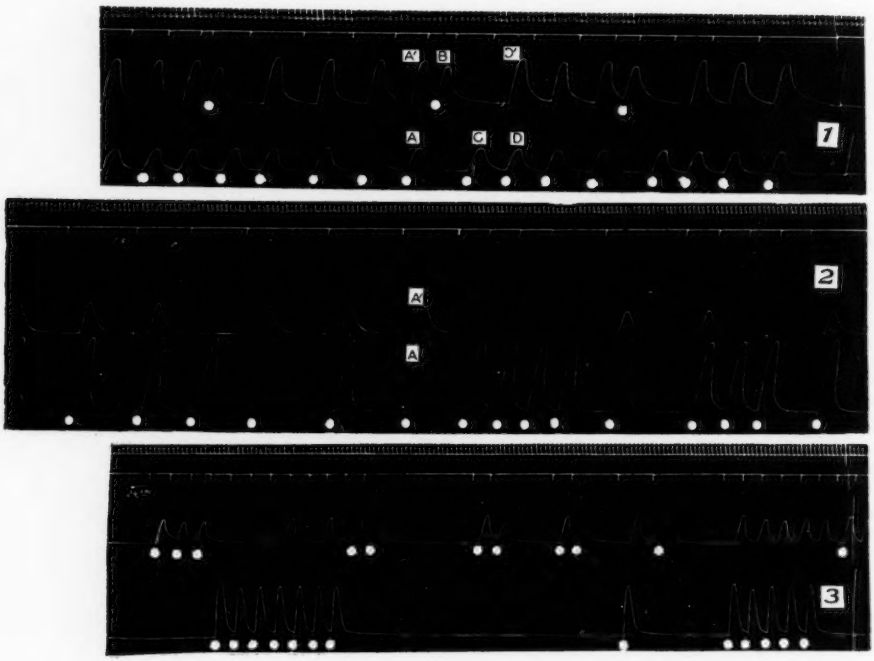

Fig. 1. Block due to refractoriness of distal segment. Tracing from turtle ventricular strip subjected to 30 cm. mercury pressure in a cuff 1 cm. wide. In order from above downward tracings represent time intervals of one second, the moment of application of shock to strip, and contractions of the two ends of the strip respectively. The segment of the strip stimulated by each shock is indicated by the presence of the white dots.

Fig. 2. Block due to insufficient time allowed for recovery of proximal segment of strip.

Fig. 3. Monodromia resulting from asymmetrical pressure injury to strip.

ever, the immediately following, blocked, but normally recovered impulse (C) of the lower segment was blocked, not because of any subnormality on its own part, but because of the relatively refractory state of the upper part. Block in this case is caused, therefore, by *failure of an impulse from a normally recovered proximal segment to stimulate the relatively refractory distal segment.*

In figure 2 are shown curves from a strip in such a condition that impulses from lower to upper portions were conducted with a 1:1 rhythm when the stimulus was applied once every 15 seconds. If the stimuli were separated by an interval of only 12 seconds, a 2:1 rhythm developed. However, following response A-A′, a 12 second interval was succeeded by three 6 second intervals and these by a 12 second interval before a conducted response was elicited. There thus occurred a period of approximately 42 seconds without a conducted response of the upper segment. Recovery of the upper segment was such that an impulse could have been propagated if the lower part had been rested for 15 seconds. However, the fact of allowing the lower segment only a short (6 seconds) intersystolic interval appears to have reduced its strength of impulse to a degree such that the impulse was inadequate to pass the blocking region and produce a response of the upper segment. However, the longer rest period allowed the upper segment results in its becoming responsive to an impulse propagated from the lower segment after a rest period of only 12 seconds for the latter. *An impulse from a more thoroughly recovered proximal segment of the strip is thus shown to have greater exciting ability than an impulse conducted in less completely recovered tissue.*

Block may occur, therefore, either because refractoriness of the distal portion of the preparation makes subthreshold a normally strong impulse conducted from the proximal segment, or because a premature response of the proximal segment gives rise to a subnormally strengthened impulse which is inadequate to cause stimulation across the junction of an even normally recovered distal segment.

It is thus seen that either an increase of threshold of the distal segment or a decrease of impulse strength of the proximal segment may cause block. In a symmetrically compressed strip, conduction times in one and the other directions are similar. Under an asymmetrical clamp, conduction in one direction requires a time which is usually much longer than that required for conduction in the other direction. The limiting case of the condition of heterodromia is, of course, a complete monodromia. With such an asymmetrical clamp as that used by Ashman and Hafkesbring, it appears that the "strength of impulse" on one side of the clamp is usually depressed to a relatively greater degree than is the threshold of the same tissue to a conducted impulse. In our own experiments, when the cuff had bellied out toward one side in consequence of too high an applied pressure, unidirectional conduction frequently developed. Usually it occurred in the manner found by Ashman and Hafkesbring, and thus essentially in accord with the hypothesis of Engelmann. That is to say, impulses were conducted *into* the segment toward which the bulging had occurred but were not propagated from that side through the blocking region to the opposite segment. Expressed in terms of depression of the tissue, it could be said

that conduction occurs in the direction of less depressed to more depressed tissue better than in the reverse direction. Figure 3, however, shows one of our records which constituted the exception to this rule. The cuff had bellied toward the lower segment and the pressure had then been removed to allow recovery. The figure is reproduced from a small part of a long continuous record. It shows first a 2:1 and then a 1:1 rhythm when conduction was in the direction of lower (more damaged) to upper (less damaged) segment. There was complete block in the opposite direction.

According to our interpretation of the facts, monodromia will occur, then, only in preparations in which an asymmetrical depression exists. Besides a mere asymmetry between the two sides of the junction, it seems probable that there must occur unequal depression of the strength of the impulse and of the excitability of the tissue at least on one side of the junction. Furthermore, there also exists a time effect coming into play in the consideration of the recovery of strength of impulse and of the recovery of excitability following a previous response. We have indirect evidence indicating that the recoveries of these two functions do not follow identical time curves. The evidence for this will be presented in another paper. Both of these functions recover during the diastolic period and their recovery curves are perhaps different expressions of the recovery of the same system. The energy required to upset the system, causing a new response, is apparently not directly proportional to the work which the system can perform when a propagated disturbance has been started. Since the manipulation separately of these functions has, so far, been found difficult or impossible of achievement, it seems likely to be a matter of practical impossibility to obtain monodromias of predictable orientation with our present knowledge.

Conclusions. Records of considerable variety have been obtained from compressed ventricular strips. Three figures are presented as indicative of our results. We believe these results to be representative of conditions obtaining in the intact heart. Therefore, if the "resistance" factor at a junction, at any time, be regarded as a constant, the passage or blocking of a propagated impulse at such a junction will depend upon two further factors. These are (1) the strength of the propagated impulse from the proximal segment, and (2) the state of excitability of the distal segment. Both of these are subnormal for a period following a previous response and recover during the intersystolic period. The times required for *complete* recovery of these two entities in a given chamber of an intact, spontaneously beating heart are similar although indirect evidence leads us to the belief that the time-recovery curves in the two cases are of different form.

In a normal heart, the cardiac impulse is more than adequate for conduction across the junctional regions. With depression of activity, however,

the margin of safety is much narrower and in a depressed preparation, a moderate increase in rate may, for example, reduce a 1:1 response to some fractionate rhythm.

The relation of these experiments to the facts relating to the occurrence and relief of partial or complete blocks in tachycardias and auricular fibrillation is obvious.

SUMMARY

Experiments have been performed with heart strips subjected to compression by a cuff 1 cm. in width. It is concluded that three factors are involved in the determination of conduction or block. The first of these factors has been referred to as resistance. It includes the complex of conditions at a cardiac junction or at the critical region of a compressed strip. Considering this as a constant in a given preparation and at a given time, the blocking or transmission of an impulse would then depend first upon the strength or the exciting power of the impulse propagated in the distal chamber or segment, and secondly, upon the state of excitability of the tissue of the distal chamber or segment into which the impulse has to be propagated across the junction or blocking region. Both of these factors increase from a minimal value to a maximal value during a time following the end of a contraction period. Sufficient depression of either of these factors independent of the other may cause block to occur, although ordinarily both are reduced together. It is assumed that it is by a relative separation of these two factors in some preparations which allows for the appearance of heterodromias or monodromias. Conduction in the normal, intact, spontaneously beating heart involves a margin of safety such that considerable increase of heart rate or of depression of the tissue may occur without the appearance of block.

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THE TIME CURVE AFTER INSULIN

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Macleod and Orr (1) have stated that it is impossible to know when the blood sugar reaches its lowest point after insulin. Some of the reasons for this difficulty are apparent from the analysis of the time curves after various doses of insulin which is given by Scott and Dotti (2). These authors have shown that, with rabbits, given a considerable number of observations, *a*, the several points on the mean time curve for a given dose of insulin may be determined with a fair degree of precision; *b*, the duration as well as the degree of hypoglycemia is related to the dosage; *c*, the curves for the lighter doses are well on their way back to the original level some time before those of the heavier doses have ceased to fall; *d*, with lighter doses at least, there may be a late hyperglycemia, occurring when the returning curve overshoots the original level. At the shortest observation period, $\frac{3}{4}$ hour, a reasonably smooth dose curve is obtained. At the next period, there has been a noticeable return toward the original level, for the lighter doses, while the points for the medium doses hold their positions and those for the heavier doses continue to fall. Scott and Dotti point out that this leads to a simulation of increased sensitivity from the fact that the full effect of the lighter doses is missed, due to their earlier recovery, and consequently their effects in relation to those of other doses is minimized. Since it has been shown by the author elsewhere (3) that the initial fall in the blood sugar level after doses of insulin may be used in the assay of insulin, it becomes desirable to explore the time curve for the purpose of determining its various characteristics and from these the optimum time to allow between the administration of the insulin and the removal of the blood samples. The purpose of this research is to determine the time curve for one relatively small dose of insulin from the time of injection until the return, or at least the approach to the original level.

Approximately 100 observations were made on each of the intervals: 20 minutes, 40 minutes, 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, 3, $3\frac{1}{2}$, 4, 5 and 6 hours following the injection of $\frac{1}{4}$ unit of insulin per kilo. Another series of 96 observations was made after 6 hours of inanition but without insulin. The purpose of this series will be explained later. Approximately 200 control observations

were made. The control animals were bled just after removal from the cages. The animals used were rats.

From the chart it will be seen that after the injection of $\frac{1}{4}$ unit of insulin per kilo, rats show a continuous drop in the blood sugar level until the 1-hour interval is reached. After 1 hour, the curve begins to return to the original level. At three hours the blood sugar has reached a level which, within small variations, remains the same throughout the remainder of the 6-hour period. It will be noted that the blood sugar does not return to the original level during the period observed. It was because of this continued depression of the blood sugar that the 6-hour inanition series was performed. As will be seen, 6 hours of starvation brings the blood sugar down to the same level as that found from 3 to 6 hours after $\frac{1}{4}$ unit of insulin.

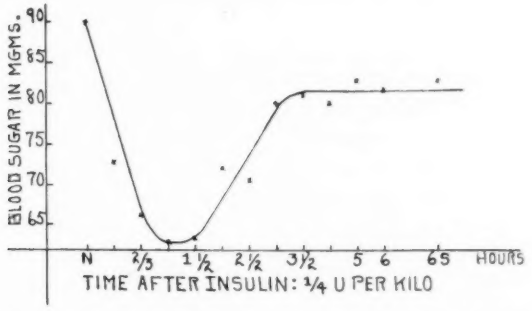

Chart shows the drop in the blood sugar level through a period of 6 hours after the injection of $\frac{1}{4}$ unit of insulin per kilo. *6S* indicates the 6-hour inanition series.

Scott and Dotti found that, with rabbits, $\frac{1}{4}$ unit per kilo produces its greatest fall in $1\frac{1}{2}$ hours. However, they took samples at only $\frac{3}{4}$, $1\frac{1}{2}$ and $2\frac{1}{2}$ hours so that the lowest point on the curve might very well have been at 1 hour instead of $1\frac{1}{2}$ hours. All of their curves for doses ranging from $\frac{1}{16}$ to 2 units per kilo, as well as the curve presented in this paper, show the fall in the blood sugar to be the most abrupt during the initial period. For example, our curve shows the greatest drop in the first 20 minutes. After 20 minutes the effect of the time is no longer proportional to the changes in the blood sugar, but becomes less and less until the lowest level is reached.

Scott and Dotti have pointed out that with a dose of $\frac{1}{16}$ of a unit there may be a late hyperglycemia. It was because of this that the observations in this experiment were continued throughout the 4th, 5th and 6th hour after injection. As will be seen from the curve, no late hyperglycemia was found. Whether this was related to the larger dose in the present

work, or whether it may have been missed due to the fact that in this region of the curve samples were taken only every hour, or whether a late rise might have been hidden by the hypoglycemia of inanition has not been determined. Whatever the reason, no late hyperglycemia was found in this series even when allowance was made for any effect of inanition. From the curve obtained, we conclude that the optimum time for taking blood samples after insulin, at least for the dose used, is $\frac{1}{2}$ hour after the injection.

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THE RELATIONSHIP OF THE UREA CLEARANCE TO THE RENAL BLOOD FLOW

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In a study of the possible causes of fluctuations in renal function, Van Slyke, Rhoads, Hiller and Alving (1934) found a parallelism between spontaneous variations in urea clearance and in the rate of renal blood flow in normal dogs.

In the present study we have tested more rigorously the extent of this parallelism by inducing maximal fluctuations in the urea clearance and observing the concurrent variations in renal blood flow. The fluctuations have been produced by diet. Jolliffe and Smith (1931) found that dogs showed regularly higher urea clearances when on high protein diets than when on low protein intake. We have confirmed this finding, and have arranged the diets to cause maximal variations in the clearance. Simultaneous observations were made of the clearance, the percentage of urea removed from the blood by the kidneys, and the renal blood flow.

EXPERIMENTAL. The experiments were performed on seven of the same dogs that were reported in the previous paper by Van Slyke, Rhoads, Hiller and Alving (1934). In each animal one kidney had been explanted by the method described by Rhoads (1934) and the other kidney removed.

In order to reduce the urea clearance to as low a level as possible the low protein diet described by Jolliffe and Smith (1931) was given for periods of 10 to 60 days. This diet contained 0.7 gram of protein per kilo per day. Between the last feeding of the low protein diet and the beginning of the observations of the clearance and renal blood flow, a fast of 40 hours was allowed. The fast appears to increase the depression in urea clearance caused by the low protein diet.

As high protein diet each dog was offered one kilo of meat daily. It was usually all consumed, although after some days some of the dogs refused part of it. In some cases the last feeding was given 1 to 2 hours before the observations of clearance and blood flow were begun. In others a period of 18 hours was permitted to elapse after the last feeding, in order to make observations in the post-absorptive period.

In order to increase the blood urea content so that the error in its determination would be minimized, and in order to insure a free flow of urine,

a solution of urea was given by stomach tube one-half hour before beginning each series of observations. The amount given per kilo body weight was 0.5 gram of urea dissolved in 12.5 cc. of water.

Each series of observations was carried through 4 successive urine collection periods of about 40 minutes each, as described by Van Slyke,

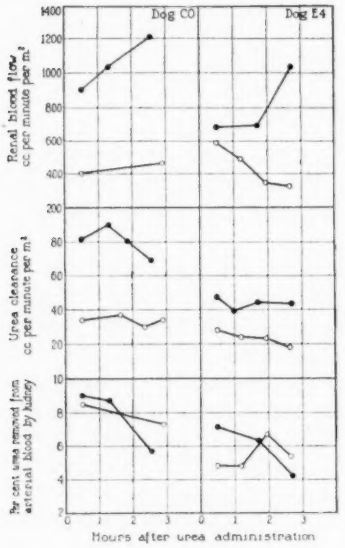

Fig. 1

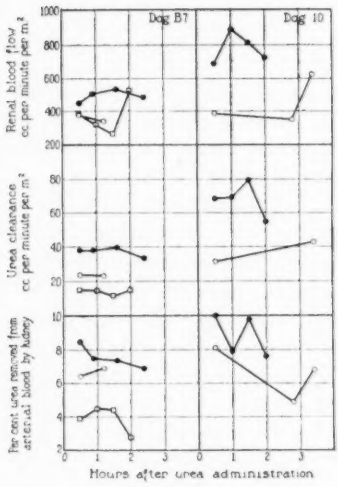

Fig. 2

Fig. 1. ○———○ Low protein diet followed by 40 hours of fasting. Low protein diet continued 10 days in dog C0 and 32 days in E4.

●———● High protein diet, with large meat feeding one to two hours before beginning of experiment. High protein diet continued 14 days in dog C0 and 9 days in E4.

Fig. 2. ○———○ Low protein diet followed by 40 hours of fasting. Low protein diet continued 34 days in dog B7 and 30 days in dog 10.

□———□ Low protein diet for 62 days followed by 40 hours of fasting.

●———● High protein diet, with large meat feeding one to two hours before beginning of experiment. High protein diet continued 8 days in dog B7 and 13 days in dog 10.

Rhoads, Hiller and Alving (1934), and involved four analyses each of blood drawn from the femoral artery and the renal vein. The arterial and renal bloods were usually drawn within 1 or at most 2 minutes of each other, and within 5 minutes of the middle of the urine collection period. The blood urea contents at the exact middle of the period were obtained by graphic interpolation on the blood urea time curves.

The *methods* for blood and urine analyses were those described in the previous papers by Van Slyke, Rhoads, Hiller and Alving (1934).

The *calculations* of urea clearance and renal blood flow were made as described in a previous paper (Van Slyke, Rhoads, Hiller, and Alving, 1934).

Results. The effect of high protein diets in increasing both urea clearance and renal blood flow to two or more times their values on very low

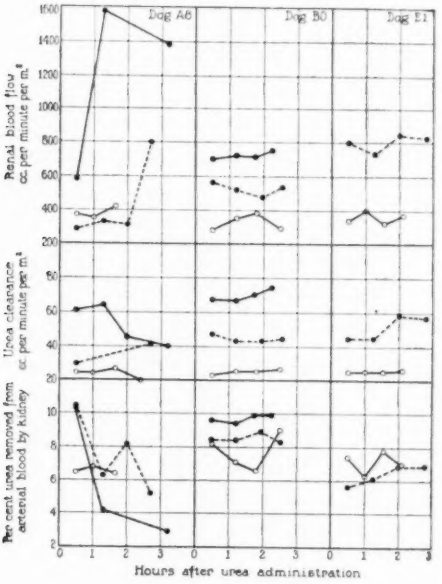

Fig. 3. ○———○ Low protein diet, followed by 40 hours of fasting. Low protein diet continued 17 days in dog A8, 15 days in dog B0, and 19 days in E1.

●— — —● High protein diet followed by 18 hours of fasting. High protein diet continued 20 days in dog A8, 17 days in dog B0 and 21 days in dog E1.

●———● High protein diet, with large meat feeding one to two hours before beginning of experiment. High protein diet continued 25 days in dog A8 and 6 days in dog B0.

protein diets in the 7 individual animals is shown in figures 1, 2 and 3. The general parallelism between urea clearance and renal blood flow is shown in figure 4.

The results confirm the conclusion of the writers (1934a) in a previous paper, that the variations in urea clearance in normal dogs are chiefly related to variations in renal flow, and only in a lesser degree, as a rule, to variations in the percentage of urea extracted from the blood by the kidneys.

Compared with the correlation between clearance and renal blood flow, the results in figure 5 show relatively little correlation between the clearance and percentage urea extraction. The high protein diets, which greatly increased the urea clearance, had no definite effect on the percentage of urea extracted from the blood by the kidneys. When the percentage urea extractions in the separate periods of each experiment of figures 1, 2 and 3 are averaged, it is found that, of the 7 dogs, 4 showed slightly higher percentages of urea removed from the blood by the kidneys when the animals

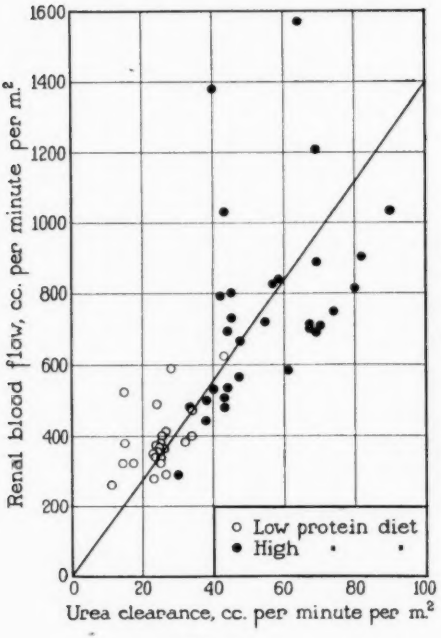

Fig. 4

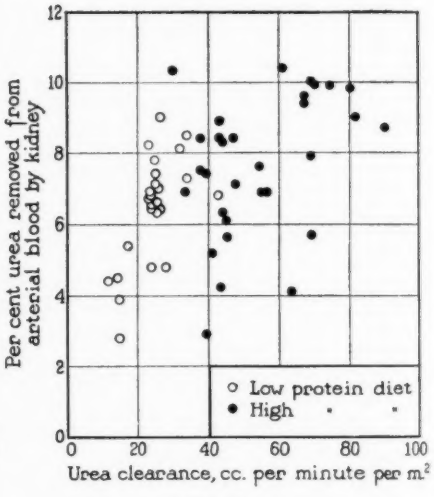

Fig. 5

Fig. 4. Relation of renal blood flow to blood urea clearance

Fig. 5. Relation of percentage blood urea removal to urea clearance

were on high protein intakes than when they were on low ones; in the other 3 animals, on the contrary, the percentage urea extractions were slightly higher on the low protein diets. Dog B-7, figure 2, showed four unusually low percentage extractions after a very prolonged low protein diet, but dog A-8 showed two similar low extraction values on a high protein diet. Figure 5 shows that when all the results are viewed there is no obvious relationship of the percentage urea extraction to either the protein intake or the urea clearance.

It appears possible that the increase in renal circulation rate which we noted in dogs shortly after ingestion of meat may be associated with an increase in the general circulation rate, since Essex, Herrick, Mann and Baldes (1934), Herrick, Essex, Mann and Baldes (1934), and Herrick, Mann, Essex and Baldes (1934) have noted increased general circulation rate after feeding.

SUMMARY

The finding of Jolliffe and Smith is confirmed, that dogs show higher urea clearance on high protein than on low protein diets.

When maximal urea clearances were obtained by meat diets, and minimal by low protein diets and fasting, variations of the clearance reaching extremes at 15 and 90 cc. per minute were obtained in a series of normal dogs.

The urea clearance was found to vary in direct proportion to the renal blood flow.

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THE EFFECTS OF NOVOCAINIZATION AND TOTAL SECTION OF THE NERVES OF THE RENAL PEDICLE ON RENAL BLOOD FLOW AND FUNCTION

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The anatomical details and physiological function of the renal urine supply have received considerable attention from a number of investigators in the past. The development of a technique for explantation of the kidney by Rhoads (1934) and the utilization of that technique for studies of the renal blood flow, urea removal, and oxygen consumption by Van Slyke, Rhoads, Hiller and Alving (1934) have made practical the reinvestigation of the problem. Furthermore, increasing interest in the possibility of the application of the procedure of renal denervation to the problems of clinical medicine warranted a reëstablishment of the experimental physiological facts upon which the procedure is based. Such experiments have been performed and are reported in this communication.

Review of the literature. The literature bearing on the subject of the nervous control of renal activity has been reviewed so frequently that it need not be discussed here in detail. Among the general reviews of the subject are those of Quimby (1916), Milliken and Karr (1925), and Caldwell, Marx and Rowntree (1931), all of which present comprehensive bibliographies.

In general the existing studies may be grouped under four main headings: The first includes simple anatomical work on the detailed structure of the renal innervation. The papers of Pappenheim (1841), Burton-Opitz (1916), Wiedhoff (1927) and Ellinger and Hirt (1925) are of this type. They may be summarized by the statement that the renal plexus is bound by fibers from the greater and lesser splanchnic nerves and from the vagus. The innervation is unilateral and the autonomic fibers accompany closely the vascular supply of the kidney even to the glomerular capillaries.

The second group of observations comprises those in which the effects of splanchnic nerve section and stimulation were studied. The earliest reported experiments of that type are those of Claude Bernard, which have been many times repeated and confirmed. In general it was found that increased urinary flow followed splanchnic section and a decreased flow resulted from splanchnic stimulation. The work of Marshall and

Kolls (1919) may be quoted as illustrative of experiments of this general type. They found that after unilateral sympathectomy there was an increased output of water, chloride, and urea on the operated side, but no change in the output of phenolsulfonephthalein or creatinine. (It appears possible that the chloride and urea increases may have been due to the increased urine volume, particularly if on the non-operated side the urine volume was below the augmentation limit for urea excretion.) The same authors in a second paper (1919) conclude that the effects of sympathectomy on excretion are the same as the effects of increase in renal blood flow, and that therefore the functional effects of sympathectomy are probably secondary to increased renal blood flow. In a third paper (1919) they show that the administration of nicotine, which paralyzes the sympathetic ganglion cells, makes the kidney on the operated side function in the same manner as the organ on the sympathectomized side. In a fourth (1919) paper they show that the kidney on the sympathectomized side reacts with a greater diuresis to administration of sodium chloride, and with about the same diuresis to sodium sulfate, as the other kidney. It was also found that compression of one renal artery limited the diuretic effect of NaCl more than that of Na_2SO_4. The results with the unilaterally sympathectomized dogs could therefore be due to increased blood flow in the sympathectomized kidney. The general conclusion from the results of Marshall and Kolls is that sympathectomy increases the blood flow of the kidney, and that the functional effects are caused by the increase in blood flow.

Richards and Schmidt (1924–25) and Bieter (1930) advanced an explanation of the experimental results just discussed. By actually observing the glomeruli of the living frog's kidney, they concluded that splanchnic section increased the number of active glomeruli, whereas stimulation of the peripheral end effected a striking decrease.

A third group of experiments are those in which all of the nerves in the renal pedicle were cut, presumably including both sympathetic and parasympathetic fibers. Such experiments have been reported by Marshall and Crane (1922), Caldwell, Marx and Rowntree (1931), Hecht (1931), and Müller (1930) with his co-workers. The results on secretory function were in general similar to those reported after simple splanchnic section. In certain instances (Richards and Schmidt, 1924) an increased patency of the glomerular capillaries was demonstrated by perfusion methods after denervation.

The fourth group of experiments includes those in which a complete section of all the renal nerve supply was insured by removing the kidney and implanting it at some other site. Among such studies are those of Lobenhoffer (1913), Quimby (1916), Dederer 1918), Holloway (1926), and Ibuka (1926). The results again are in essential agreement and indicate that the effect of the radical procedure is not different from splanchnic

section or total denervation on the pedicle. The transplanted kidneys continued to function well and to respond to diuretics.

Taken as a whole the reported results appear to provide evidence that renal denervation increases the renal blood flow above the normal, and that the denervation prevents the retarding of renal blood flow to less than normal by vasoconstricting influences. If Volhard's (1931) theory is correct, in accordance with which the primary damage to the nephritic kidney is caused by vasoconstriction and resulting ischemia, the conclusions of the experimental work quoted above make it logical to expect that denervation might relieve such constriction, and prevent the progress of the irreversible anatomical changes that destroy the organ. Volhard (1931, p. 1320) points out the possibility that the beneficial influence of decapsulation on the anuria of acute nephritis may be due to removal of nervous control of the kidney. A number of authors (cited by Volhard) have in fact practiced denervation of the kidney instead of decapsulation and have reported successful results.

EXPERIMENTAL. *Novocainization.* Each animal employed had had the left kidney explanted and the right kidney removed about two years before. The technique of the procedure has been described by Rhoads (1931; 1934).

Each experimental observation of renal blood flow and urea clearance was carried out during four successive periods of about 40 minutes each, as described in a previous paper (Van Slyke, Rhoads, Hiller and Alving, 1934a). The first one or two periods served as controls, without novocainization. Then the area around the renal vessels was injected with novocain in the amounts and at the times indicated in figures 1 and 2.

Before the experiment urea was administered by mouth, to the amount of 0.5 gram dissolved in 12.5 cc. of water, for each kilo of body weight. The purpose of the administration of urea and water was to assure a urine flow above the augmentation limit, and a blood urea content high enough to permit its determination with maximal accuracy (see account of blood analyses, Van Slyke, Rhoads, Hiller and Alving, 1934a).

In order to make certain that the renal blood flow was not already at its maximum possible rate before novocainization, each animal was kept on a low protein diet. As shown in the preceding paper (Van Slyke, Rhoads, Hiller and Alving, 1934b), the renal blood flow on this diet falls, usually to less than half its value on a meat diet.

The results of the experiments are given in figures 1 and 2.

Denervation. Each dog had had the left kidney explanted and the right one removed two years before. The denervation was carried out under full ether anesthesia. A left rectus incision was made extending distally about 10 cm. from the costal margin and continued into a transverse incision extending to the vertebral column. Thus a triangular flap was

formed which included the explanted kidney and which could be tipped up to expose the renal pedicle. All the nerve fibers were cut as they passed through the peritoneum, stripped back to their origin, cut again, and removed. The vessels and ureter were then scrubbed with dry gauze to remove any superficial fibers which might have escaped attention. The flap was then replaced and the wound closed. No untoward effects were observed to follow this procedure.

Experiments to determine the urea clearance and renal blood flow were performed before and after the denervation in each animal. For these

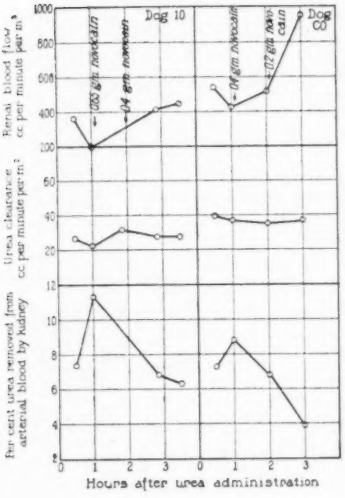

Fig. 1

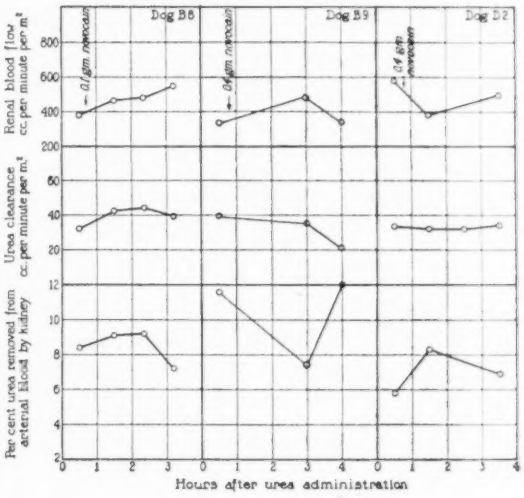

Fig. 2

Fig. 1. ○———○ Experiments showing effects of blocking renal nerves with novocain. Each dog was on low protein diet followed by 40 hours of fasting. Dog 10 on diet 43 days; dog C0 32 days.

Fig. 2. ○———○ Experiments showing effects of blocking renal nerves with novocain. Each dog was on ordinary kennel diet, followed by 24 hours of fasting.

experiments the technique of Van Slyke, Rhoads, Hiller and Alving (1934a) was again employed.

The animals were varied from high to low protein diets in order 1, to show that the kidneys were normally elastic with respect to their ability to vary their blood flow, and 2, to set the renal blood flow immediately before denervation at a minimal level, so that any accelerating influence of denervation on the blood flow might show a maximal effect.

The results are shown in figure 3.

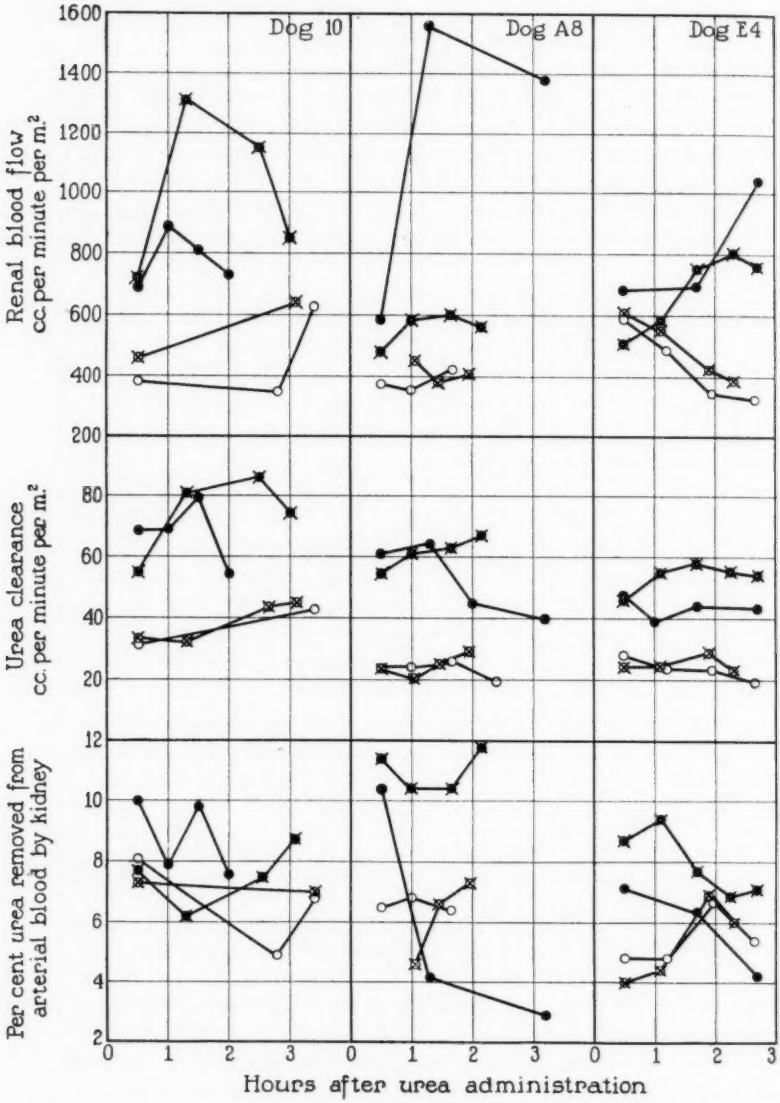

Fig. 3. ○———○ Low protein diet followed by 40 hours of fasting, *before* denervation.

⊠———⊠ Low protein diet followed by 40 hours of fasting, *after* denervation.

●———● High protein diet with large meat feeding one to two hours before beginning of experiment, *before* denervation.

◙———◙ High protein diet with large meat feeding one to two hours before beginning of experiment, *after* denervation.

DISCUSSION. The data in figures 1, 2, and 3 show a complete failure of the experimental procedures to effect any consistent alteration of either the renal blood flow or of the renal function, as measured by the urea clearance. These results are in striking contrast to previous work on the subject. Although an adequate explanation of the discrepancy is not at hand certain features of the experiments deserve particular consideration.

Since the studies were made on animals from which one kidney had been removed some time previously, the organ investigated was one which had presumably undergone physiological hypertrophy. That such was actually the case was shown in former papers by the writers (Rhoads, Alving, Hiller and Van Slyke, 1934; Van Slyke, Rhoads, Hiller and Alving, 1934). Hence the explanation might be offered that failure to increase the renal circulation by novocainization or section of the nerves on the renal pedicle might conceivably be due to the presence of a maximum degree of vasodilatation already before the nerves were blocked or cut. That this explanation is not the true one, however, becomes clear from the fact that in spite of the physiological hypertrophy the renal circulation responded normally to low and high protein intakes, proof that the renal vessels were still under control, and that on the low protein diets, at least, the renal circulation rate was far short of maximal.

A second explanation which might be advanced is that total denervation was done rather than simple splanchnic section. Hence, presumably, both constrictor and dilator fibers were cut, and the end result might be a failure to affect either constriction or dilatation by the procedure employed. Two facts stand opposed to this hypothesis: first, that previous studies have shown no difference in the effects produced by total denervation from those of simple splanchnic section, and second, that vaso-constrictor fibers in the vagus have never been unequivocally demonstrated.

Another possible explanation of the divergent results may be based upon the fact that the procedures employed both as regards the experimental animals and the methods of estimating blood flow and urea excreting power differed radically from the procedures by which previous workers in the field have deduced conclusions concerning renal blood flow and function.

CONCLUSIONS

Denervating the kidneys of dogs, or blocking the renal nerves with novocain, was without consistent effect on either the excretory efficiency of the kidney, as measured by the urea clearance, or on the renal blood flow.

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THE ELECTRICAL EXCITABILITY OF THE NICTITATING MEMBRANE

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Autonomic neuromuscular systems have a crucial significance with regard to Lapicque's (1926) theories of isochronism and curarization, a significance which has been neglected. Lapicque and his collaborators (see 1926 for references) recognize heterochronism in autonomic systems, but correlate it with the "iterativeness" of these structures. They postulate that in such systems a series of nerve impulses would be necessary to obtain any muscular response. Iterativeness in autonomic neuroeffector systems is far from being the rule, however, as is shown by the work of Gilson (1931), Bishop and Heinbecker (1932), Rosenblueth (1932), and Rosenblueth, Forbes and Lambert (1933). It was therefore deemed of importance to investigate the isochronism or heterochronism of the nictitating membrane, a non-iterative system of the cat. This system is especially suitable for such observations because of its single sympathetic nerve supply (Rosenblueth and Bard, 1932). As will be shown in the discussion, the results obtained have a bearing on other aspects of the physiology of smooth muscle and on the general problem of excitability.

METHOD. Cats were used, under dial anesthesia. Isotonic contractions of the nictitating membrane were recorded by a light lever giving approximately a 15-fold magnification.

The stimuli used were discharges from condensers of variable capacities and intensities, applied either singly or repetitively at a constant frequency determined by a metronome. Variable resistances were connected in series and in parallel with the stimulating electrodes. Single waves of direct current of variable durations (Lucas pendulum) and intensities were used in some instances to control the results obtained by single condenser discharges and to determine rheobases. The electrodes were fine silver-plated and chlorided needles (diameter 0.5 mm.). The cathode was inserted at the free margin of the nictitating membrane immediately below the serrefine connecting the muscle with the recording lever and the anode was placed subcutaneously in the nose or forehead.

Cocaine (about 8 mgm. per kgm.) was injected intravenously for the observations on responses to single shocks, in order to increase these

responses (Rosenblueth and Rioch, 1933a). Curare was injected to prevent perturbing contractions of neighboring skeletal muscles.

In all experiments, progressively increasing, progressively decreasing and random stimuli were applied.

RESULTS. A. *Distribution of voltage thresholds.* The nictitating membrane was stimulated directly as described above. The capacity of the condenser was maintained constant while various voltages were applied. Whether single shocks or repetitive stimuli at a constant frequency were employed there appeared invariably a break in the distribution curves which correlate the responses with the voltages (R-V). Figure 1 illustrates typical results with repetitive stimulation; they are closely similar

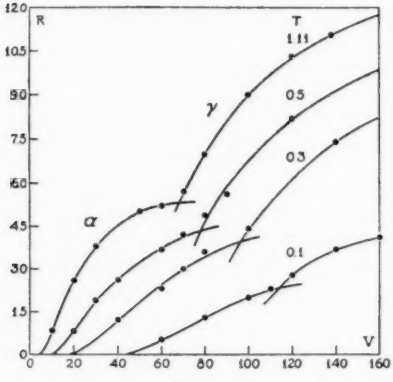

Fig. 1

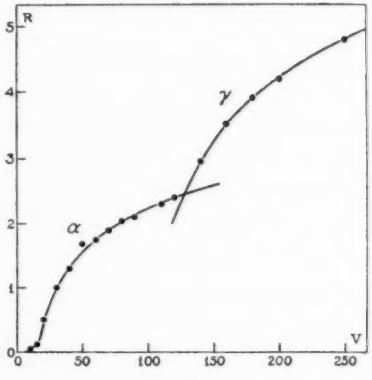

Fig. 2

Fig. 1. Dial, curare and cocaine. Repetitive stimulations at a frequency of 2 per sec. Ordinates: height of responses in centimeters in the record. Abscissae: volts. Indexes: capacities in μ F.

Fig. 2. Dial, curare and cocaine. Single condenser discharges with a capacity of 0.5 μ F. Ordinates and abscissae as in figure 1.

to those obtained by single shocks in figure 2. The level at which the break occurs is a function of the condenser capacity (in μF) or the duration of the stimuli (figs. 1 and 8); the shorter the stimuli, the smaller the response and the higher the voltage at which the breaks appear.

B. *Distribution of duration thresholds.* A constant voltage was applied at various durations and the corresponding responses were plotted against these durations. Again there appeared invariably breaks in the distribution curves (R-T) thus obtained. Figures 3 and 4 illustrate typical results for repetitive and single stimuli, respectively. The level of the breaks was found to be a function of the voltage, occurring at lower durations and responses for higher voltages (figs. 4 and 9).

C. *Voltage-duration curves.* These were obtained by the method of interpolation described by Rosenblueth and Rioch (1933b). Within a given range of responses, families of curves such as that represented in figure 1 were recorded. These families were then slabbed horizontally at various levels comprised within the selected range of responses and the durations corresponding to the intersected curves were plotted against the corresponding voltages at the intersection. The curves then represent the voltages and durations which furnish a constant response, i.e., since the frequency was constant, the voltages and durations which activate the same number of elements to a similar degree. Whether repetitive or

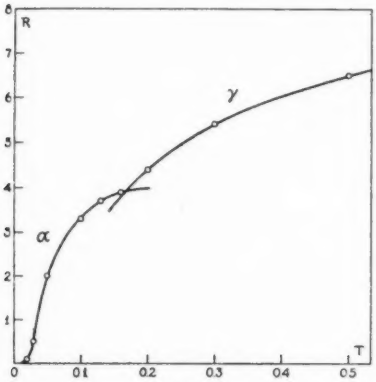

Fig. 3

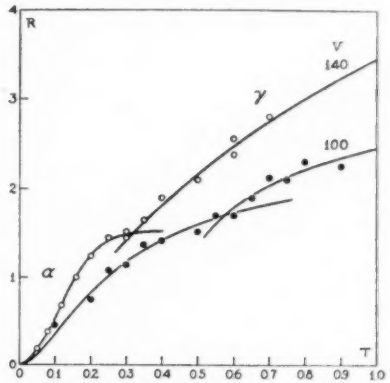

Fig. 4

Fig. 3. Dial, curare and cocaine. Repetitive stimulations at a frequency of 2 per second and with an intensity of 80 volts. Ordinates: height of responses in centimeters in the record. Abscissae: capacities in μ F.

Fig. 4. Dial, curare and cocaine. Single condenser discharges. Ordinates and abscissae as in figure 3. Indexes: volts.

single stimuli were employed the voltage-duration (V-T) curves thus obtained presented distinct breaks.

Figure 5 (dots) and figure 6 illustrate typical results. Following a general convention we shall designate the upper and left branch of the V-T curves as γ and the lower and right as α (Lucas, 1907). Since the present curves were obtained by horizontal slabbing of R-V or R-T families we have extended this designation of α and γ to the corresponding segments in the original curves (figs. 1, 2, 3 and 4).

The level of the break at which the transition from α to γ occurs in the V-T curves was found to be a function of the response selected for slabbing (cf. fig. 10); the greater the response, the longer the duration at which the break appears.

D. *Quantity-duration curves.* These were obtained by plotting the products VT against the corresponding T. A break in the approximately straight lines thus obtained (Weiss' formula, see below) marked again the transition from α to γ (fig. 5, circles, and fig. 7).

E. *Excitation times.* The excitation times (E. T.) of the γ excitability (Lucas, 1907) in the V-T curves (section C) could not be determined directly because of the uncertainty of the rheobase. From the VT-T curves (section D), however, a close approximation of the rheobase may be obtained using Weiss' formula $V = a + b/t$, where a is the rheobase. The slope of the straight lines in figures 5 and 7 determines then a.

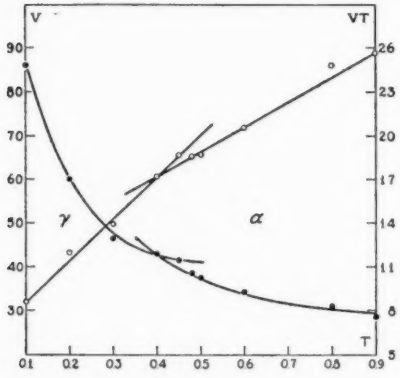

Fig. 5

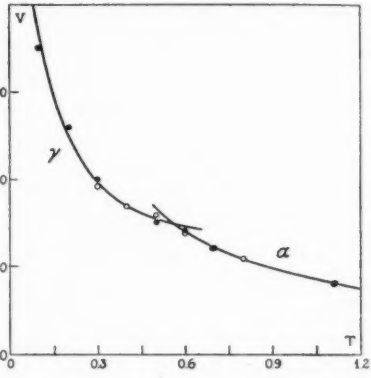

Fig. 6

Fig. 5. Dial and curare. Repetitive stimulation at a frequency of 3 per second. R-V family (see text) slabbed at 4 cm. (about 50 per cent of the maximal response). Ordinates: volts (left scale, dots) and quantities (V T, right scale, circles). Abscissae: capacities in μ F.

Fig. 6. Dial and curare. Repetitive stimulation at a frequency of 2 per second. Ordinates: volts. Abscissae: capacities in μ F. Dots: observations made before cocaine. Circles: observations made after injecting 8 mgm. of cocaine per kilo intravenously. See text for levels of slabbing of the R-V families.

The E. T. of the α curves was approximately $0.75\sigma \pm 0.2$ (average of 11 curves obtained from 6 animals). The E. T. of the γ curves, determined as described in the preceding paragraph was $0.3\sigma \pm 0.1$ (average of 15 curves obtained from 9 animals). The latter figure coincides with the chronaxie of the nerve supply (cf. Rosenblueth and Rioch, 1933b).

F. *Effects of curare and cocaine.* Since these drugs were employed in the experiments it was considered necessary to examine their effects on the α and γ excitabilities. The V-T curves obtained before and after injections of curare were practically identical for a given response (within less than 10 per cent).

Cocaine increases the responses to any given submaximal stimulus (cf. fig. 11). The results obtained before and after its injection are therefore not directly comparable. The following procedure was employed to determine its action. A stimulus was selected which elicited before cocaine precisely the response at which the R-V family was slabbed. The same stimulus was then applied after cocaine and the new R-V family was slabbed at the level of the new higher response. It was then found that the two V-T curves superimposed satisfactorily, as shown in figure 6. The coincidence of the VT-T curves corresponding to figure 6 is shown in figure 7.

We may therefore conclude that neither curare nor cocaine has any significant effect on either the α or the γ excitabilities. As will be shown

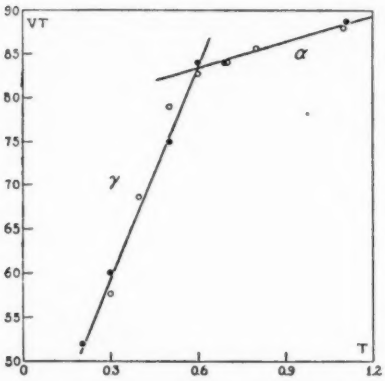

Fig. 7

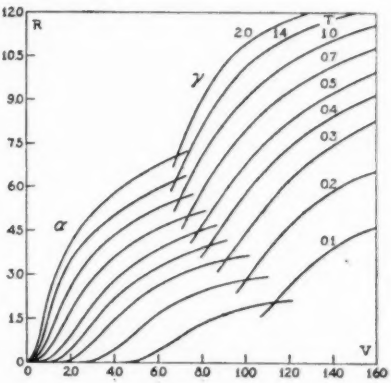

Fig. 8

Fig. 7. As in figure 6, but ordinates: quantities.

Fig. 8. R-V family. Ordinates: responses. Abscissae: volts. Indexes: capacities. For explanation see text.

in the discussion the lack of effect of cocaine in altering the time parameters of the V-T curves is of interest when contrasted with the striking effects the drug has on the contraction time of the nictitating membrane. Figure 11 illustrates the influence of cocaine on the responses; their height and duration are increased.

G. *Responses to α or γ stimuli.* The responses to stimuli belonging to a γ or an α segment of the V-T curves are indistinguishable in shape, rate of contraction and rate of relaxation. Figure 12 illustrates their similarity.

H. *The total distribution of thresholds in the neuro-muscular system.* This distribution will be depicted graphically by complete sets of R-V, R-T and V-T curves. Only one of these need be determined experimentally. The other two will then be readily obtained by adequate slabbing

of the experimental family (Rosenblueth and Rioch, 1933b), for the three variables are mutually interrelated.

It is, however, difficult to obtain a complete experimental family with accuracy, because of the large number of observations required. There occurs usually a progressive decline in the responses to any given stimulus, especially noticeable for responses to α stimuli. This decline may not be significant for a relatively limited number of observations such as those plotted in figures 1 to 7. But it would definitely influence the aspect of the complete experimental family of curves in question.

In constructing the families reproduced in figures 8, 9, and 10, the following procedure was employed. The curves indexed 1.0, 0.5, 0.3 and

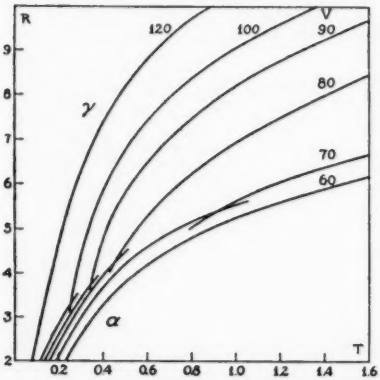

Fig. 9

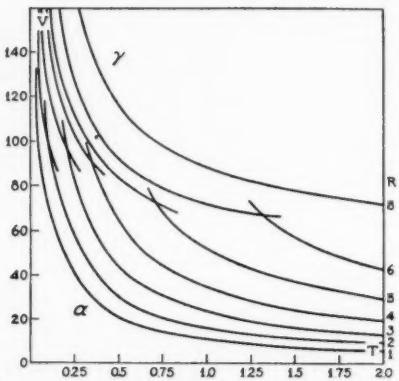

Fig. 10

Fig. 9. R-T family. Ordinates: responses. Abscissae: capacities. Indexes: volts. For explanation see text.

Fig. 10. V-T family. Ordinates: volts. Abscissae: capacities. Indexes: responses. For explanation see text.

0.1 in figure 8 were obtained experimentally. These were then slabbed horizontally to yield V-T curves. The latter were next plotted as VT-T lines, which may be taken as straight, a slight error being committed only for very short or very long durations. From these straight lines new V-T points could be read completing the V-T family in figure 10. Finally vertical slabbing of the V-T family completed the R-V family in figure 8 and either horizontal slabbing of figure 10 or vertical slabbing of figure 8 led to the R-T family in figure 9. The accuracy of the R-V curves was further examined by plotting in logarithmic probability paper, which yields practically straight lines.

Discussion. Complex V-T curves, similar to those obtained in the experiments reported above (figs. 5 and 6) have been interpreted to repre-

sent two different excitabilities in skeletal muscle, that of nerve (γ) and that of muscle (α). The nerve would thus possess a shorter excitation time (in the sense of Lucas, 1907) than the muscle.

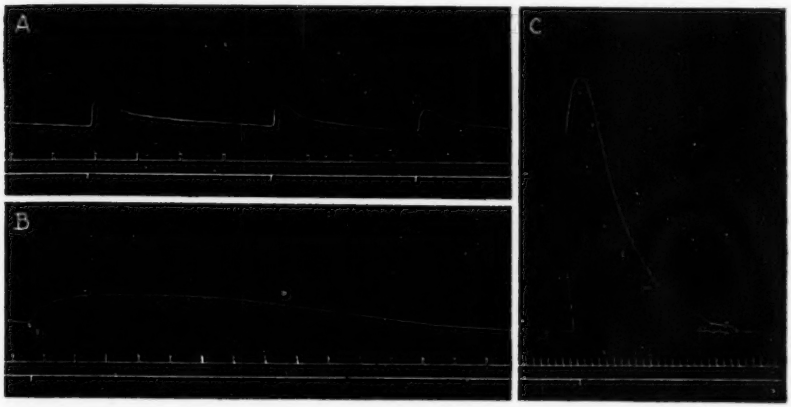

Fig. 11. Dial. Electrodes on cervical sympathetic cut centrally. Stimulation by single condenser discharges. Time recorded in 5-second intervals. The speed of the kymograph was slowed before each successive record.

A. Before cocaine.

B and C. After injection of 8 mgm. cocaine per kilo.

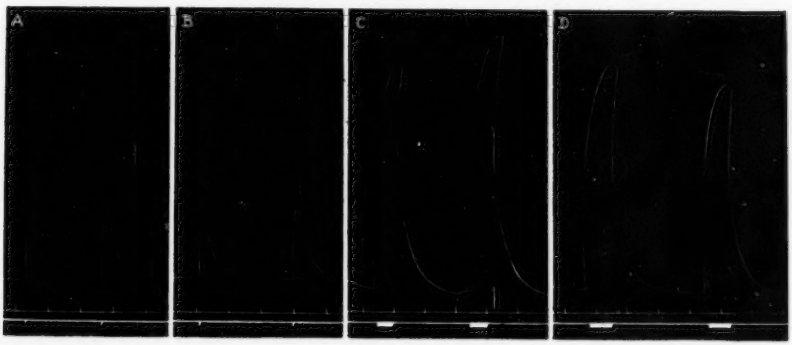

Fig. 12. Dial, curare and cocaine.

A. Responses to single α stimuli. Capacity: 0.5 μ F. Voltages: 70 and 80.

B. Responses to single γ stimuli. Capacity: 0.4 μ F. Voltages: 100 and 130.

C. Responses to repetitive α stimuli. Capacity: 1.11 μ F. Voltages: 20 and 30.

D. Responses to repetitive γ stimuli. Capacity: 0.5 μ F. Voltages: 100 and 90.

This interpretation, which was suggested by Lucas (1907) and recently defended by Rushton (see 1933 for references) has been contested by

Lapicque (see 1934). Lapicque argues that the α excitability in striped muscle is an artifact, due to the large size of the electrodes employed (Ringer bath). Such electrodes would produce a peculiar type of polarization which he calls retrograde polarization. That is, the α curve would be that of muscular electrical excitability, but not physiological, because deformed by this polarization.

Retrograde polarization would be a function of electrode size and negligible in the case of striated muscle if the electrodes are smaller than 0.5 mm. (Lapicque, 1934). The results reported here can then not be due to retrograde polarization, for the real electrodes—i.e., physiological—stimulating smooth-muscle fibers can not be larger than the muscle cells themselves, and these cells are smaller than the limit of 0.5 mm. fixed by Lapicque. Retrograde polarization due to large electrodes might, furthermore, not be the cause of the α excitability of skeletal muscle, for Bonnardel and Liberson (1934) have shown that it may frequently appear with stigmatic electrodes.

Another argument stressed by Lapicque (1933) against the physiological significance of the α excitability is that it does not vary with the rapidity or sluggishness of various muscles as judged by their contraction times (total duration of the single twitch). The γ excitability, on the other hand, would show a definite correlation with the contraction time. Lapicque quotes the following figures for three different systems:

	α E. T.	$\gamma\,\tau$	C. T.
Frog, striped muscle	10–20σ	0.3σ	0.1–0.2 sec.
Turtle, striped muscle	10–20σ	2–3σ	1.5–2.0 sec.
Holothuria, smooth muscle	10–20σ	9σ	6–8 sec.

(α E. T. = α excitation time; $\gamma\,\tau$ = γ chronaxie; C. T. = contraction time.)

To the above list we shall add the figures for the cat's nictitating membrane:

	α E. T.	$\gamma\,\tau$	C. T.
Nictitating membrane:			
Without cocaine	0.75σ	0.3σ	10 to 20 sec.
After cocaine	0.75σ	0.3σ	60 to 120 sec.

The $\gamma\tau$ is much too small in relation to the C. T. when compared with the values given above for frog's and turtle's striped muscle. The same criticism holds for the γ excitability, therefore, as has been applied by Lapicque to the α curve. Similar discrepancies appear in some of the figures ob-

tained in Lapicque's laboratory (cf. crab's claw and gastrocnemius of Calyptocephalus in the data compiled by Fredericq, 1928, p. 516).

These discrepancies, however, need not be surprising. There is no *a priori* reason why excitation and contraction times should be mutually interrelated, i.e., be functions of each other. According to current ideas, excitation time will depend on the factors that affect the rate of depolarization of the tissue, e.g., degree of polarization, electrical resistance of the system, velocity of destruction or removal of the ions at the interphase, etc. Contraction time, on the other hand, will be affected by a different set of variables, e.g., the visco-elastic properties of the muscle, the rates of production, diffusion and destruction of the chemical mediator in smooth muscle, etc. It is then not astonishing that the two times should vary independently in different muscles. The action of cocaine on the nictitating membrane emphasizes this independence: the excitation times are practically the same after injection of the drug (fig. 6) while the contraction time becomes strikingly longer (fig. 11). This duration depends on the magnitude of the contraction, but even for equal heights the responses are longer after than before cocaine (fig. 11).

Other discrepancies between excitation time and various characteristics—including excitability—of the excitable structures have been reported. Thus Rosenblueth and Rioch (1933b) found a higher chronaxie for the most excitable nerve fibers in the sympathetic supply of the nictitating membrane and in the vagal decelerator supply of the heart, as judged by the complete intensity-duration curves, than for less excitable fibers closer to the mean excitability in the nerves studied. Similar results were reported by Blair and Erlanger (1933) for the frog's nerves. Blair and Erlanger further studied conduction rate, intensity thresholds, recovery rates, amplitude of axon potentials and time to maximum of the spike potential, and concluded that chronaxie is not correlated with these characteristics. The results of Lambert, Skinner and Forbes (1933) are likewise of importance in this connection. After section of the motor nerve, the V-T curve shifts to the left and down, denoting an increased excitability, while the chronaxie increases, denoting a decreased excitability. We are therefore led to conclude that the chronaxie of a tissue cannot be taken as the chronological index "governing most, if not the whole, of the vital functions in the sphere of excitation" as Lapicque (1934) would have it. A suitable measure of excitability remains to be found.

From the preceding argument we infer that the α component in the V-T curves denotes a physiological excitability different from the γ excitability, which is similar to that of nerve. The breaks in the R-V and R-T curves for the distribution of thresholds (figs. 1, 2, 3 and 4) lead likewise to the conclusion that we are dealing with two different excitabilities. These curves were found to be smooth and continuous for multifibered nerves

(Rosenblueth and Rioch, 1933b). If there is a nerve component in the present distribution curves, as is probable, it is then reasonable to attribute the breaks to some other excitable element differing from the corresponding nerves not only in the time parameter, but also in the voltage parameter. The most satisfactory explanation available appears to us to be that offered by Lucas (1907) and adopted by Rushton (1933) and Delville (1934), that the excitability of muscle differs from that of nerve—i.e., that the two structures may be heterochronic. Physiological heterochronism of nerve and striped muscle has likewise been postulated by Brücke (1867) and Grundfest (1932). It is interesting to note that non-iterative conduction in a heterochronic system has been reported from Lapicque's laboratory for the heart. The ratio of the chronaxies of the auricle or ventricle to that of the bundle of His has been found to be 1:3 (see Fredericq, 1928, p. 516).

As stated previously, Lapicque (1926) suggested that heterochronism in autonomic systems could be overcome by reiteration of the nerve impulses. In skeletal muscle, however, repetitive nerve stimulation may not overcome the barrier interposed by curare which would merely induce heterochronism (Lapicque, 1934). This difficulty in the interpretation disappears if non-iterative heterochronic conduction is accepted and the action of curare is explained otherwise.

Changes in the extent and rate of threshold contractions in skeletal muscle when passing from the α to the γ excitabilities have been described by Bonnardel and Liberson (1934) and Bonvallet and Néoussikine (1934); unfortunately no records are published. In Rushton's (1932) and in the present experiments (fig. 12) the mechanical responses to the two excitabilities are indistinguishable. It is therefore probable that the same effector cells were activated in a similar manner with both α and γ stimuli.

It was previously reported that the denervated nictitating membrane appears to be electrically inexcitable (Rosenblueth and Cannon, 1934). This view was difficult to reconcile with the presence of a muscular initial spike potential in the electrogram obtained on stimulation of the nerve supply (Rosenblueth, Leese and Lambert, 1933), since the generally accepted membrane theory correlates the two phenomena—spike potential and electrical excitability. This difficulty disappears with the inferences from the present study. The muscular electrical excitability exists in the conditions in which the spike potential is obtained, i.e., with the nerves present.

SUMMARY

The innervated nictitating membrane of the cat was stimulated by means of condenser discharges of various capacities and intensities.

The distributions of voltage thresholds (capacity constant, figs. 1, 2

and 8) and of duration thresholds (voltage constant, figs. 3, 4 and 9) and the voltage-duration curves (response constant, figs. 5, 6 and 10), all presented breaks denoting two components: γ and α. The quantity-duration curves (figs. 5 and 7) likewise presented breaks. The average excitation times were 0.3σ for the γ and 0.75σ for the α component.

The arguments presented by Lapicque against the physiological significance of the α excitability of skeletal muscle are discussed. It is concluded that in the present experiments the size of the electrodes is not responsible for the α excitability (p. 406), that there is no correlation between chronaxie and contraction time (p. 407), that the α excitability is physiological and corresponds to the muscle (p. 408), and that nerve and muscle may be heterochronic in a non-iterative system (p. 408).

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THE UTILIZATION OF THE CALORIGENIC ACTION OF DIIODOTHYRONINE AND THYROXINE IN MUSCULAR EXERCISE

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It was shown by one of us (Rapport, 1929) that the calorigenic ("specific dynamic") action of tyrosine could not be used to do muscular work; in other words, that the "waste heat" incidental to its metabolism in the body remained waste heat during exercise as well as at rest. In this respect, tyrosine behaved like protein, but unlike carbohydrate and fat. In the present communication, we have extended our observations to the comparable effects of thyroxine and diiodothyronine.

METHODS. These were essentially similar to those of the above mentioned research. The experiments were conducted on two well-trained dogs subsisting on a maintenance diet of about 60 per cent carbohydrate, 25 per cent fat, and 15 per cent protein. In the "basal" experiments, the resting metabolism both before and after exercise was obtained in a previously described closed system for obtaining the gaseous exchange. Exercise consisted of 15 minutes' running at about 2.6 kilometers per hour on a horizontal treadmill introduced into the system; and the metabolism of exercise and recovery calculated by computing the amount of O_2 intake and CO_2 output in excess of that at rest, averaging the pre- and post-exercise resting metabolism.

Thyroxine (Hofmann-LaRoche) and diiodothyronine (Schering-Kahlbaum) were injected intravenously, except in one case when diiodothyronine was given subcutaneously. The thyroxine was injected in 1:1000 solution as bought; diiodothyronine was dissolved in N/10 NaOH, making a 2 per cent solution having a pH of 10.9. No exercise was performed (with one exception—see table 1) on the day the substances were injected, in the first place because we wished to determine, for purposes extraneous to the present communication, the time of onset of the calorigenic action; in the second place because it is difficult to calculate the excess metabolism of exercise unless the resting metabolism is practically constant. On succeeding days after injections, the resting gaseous exchange was determined over sufficiently long periods of time to establish the level, and the excess metabolism of exercise computed as above.

During the course of the work, 8 alcohol checks were performed, the average R.Q. being 0.661, a mean error of 0.8 per cent; the maximum deviation being 0.655, an error of 1.7 per cent.

Throughout, the term "work" is used loosely, signifying weight of animal moved through horizontal distance.

EXPERIMENTAL RESULTS. I. *Experiments on dog 18.* a. *"Basal."* The "basal" experiments included the conventional resting metabolism in the post-absorptive state, and the metabolism of exercise under the same conditions. They are marked "A" in the tables. As will be seen in table 1, the average "basal" resting metabolism in 9 experiments was 14.3 Cals. per hr., with a maximum variation of 0.5 Cal. Under these "basal" conditions, the dog required an average of 0.85 gram cal. of excess metabolism per kgm. of work, the highest energy consumption being 0.94 gram cal. on January 15, 1932.

b. *Thyroxine.* After giving 1.5 mgm. per kilo of thyroxine intravenously the calorigenic action of the substance was 284 Cals., and lasted 144 hours, the average increase in metabolism on the days on which exercise was performed, noted in table 1, being 17 per cent. The extra metabolism due to exercise on these days has been calculated as the excess above the "basal" resting level. This is simply a matter of choice, as it might have been equally well calculated as the excess above the resting level of the same experiment. Had this been done, it would have shown the gram cals. per kgm. of work to have been slightly higher than the figures of the same column in the "basal" experiments, and the conclusions drawn would of course have been the same. The energy consumption in exercise for the thyroxine experiments were 1.15 cals. per kgm. of work; in other words, an increase of 35 per cent over the extra metabolism of exercise and recovery in the "basal" experiments. As the specific dynamic action of the substance was 17 per cent, it is clear that not only was there summation of the work metabolism and this extra heat of the specific dynamic action, but also a large excess. We will discuss this presently.

c. *Diiodothyronine.* The intravenous administration of 15.0 mgm. of this substance per kilo resulted in a rise in the resting metabolism lasting about 144 hours, the total increase being 280 Cals., and the average calorigenic action being 17 per cent of the basal metabolism (table 1). As in the case of thyroxine, the energy consumption during exercise and recovery was greater than under "basal" conditions, this excess amounting to an average of 27 per cent.

II. *Experiments on dog 27.* a. *"Basal."* In spite of an increase in weight during the course of the experiments, this well-trained animal did not appreciably vary her basal metabolism, which averaged 11.5 Cals. per hour (table 2). Her energy consumption per kilogram of work under these conditions averaged 1.14 gram cals. With one exception, when this

TABLE 1

Dog 18 (weight about 11 kilos)

DATE	URINE N PER HOUR	RESTING METABOLISM PER HOUR*		INCREASED RESTING METABOLISM OVER "A"	WORK†	EXCESS METABOLISM OF EXERCISE‡			INCREASED EXERCISE METABOLISM OVER "A"
		R.Q.	Cals.			R.Q.	Cals	Gm. cals. per kgm. of work	
Basal "A"									
1931	*mgm.*			*per cent*	*kgm.*				*per cent*
Dec. 17	80	0.85	14.4		6988	0.92	5.2	0.74	
Dec. 21	70	0.85	13.8		7100	0.81	5.2	0.73	
Dec. 31		0.80	14.5		7026	0.79	5.8	0.83	
1932									
Jan. 15	76	0.85	14.3		7564	0.88	7.1	0.94	
Jan. 16		0.87	14.9		7434	0.85	6.6	0.89	
Jan. 18	79	0.87	14.3		7405	0.86	6.6	0.89	
Jan. 19		0.85	13.8		7315	0.93	6.4	0.87	
Jan. 26	82	0.85	14.3		7200	0.72	6.2	0.86	
Average.....		0.85	14.3			0.85		0.85	
Thyroxine, January 1, 1.5 mgm. per kilo, intravenous									
Jan. 2	90	0.81	17.3	21	7212	0.71	9.4	1.30	53
Jan. 3	98	0.76	17.6	23	6979	0.74	9.4	1.35	59
Jan. 4	60	0.81	16.3	14	6882	0.81	7.7	1.12	32
Jan. 5	73	0.83	16.1	13	7091	0.83	6.7	0.94	11
Jan. 6	80	0.84	16.1	13	7157	0.91	7.4	1.04	22
Average.....		0.81	16.7	17		0.80		1.15	35
Diiodothyronine, January 20, 15.0 mgm. per kilo, intravenous									
Jan. 20	55	0.86	17.9	25	7216	0.87	8.6	1.19	40
Jan. 21	71	0.78	17.5	22	7117	0.76	8.2	1.15	35
Jan. 22	114	0.80	17.6	23	7214	0.78	8.2	1.14	34
Jan. 23	95	0.80	16.4	15	7179	0.85	7.8	1.09	28
Jan. 24	82	0.79	15.2	8	7150	0.79	6.7	0.94	11
Jan. 25		0.82	15.6	9	7193	0.89	6.8	0.95	12
Average.....		0.81	16.7	17		0.82		1.08	27

* Average of pre- and post-exercise resting metabolism.

† Running on a horizontal treadmill for 15 minutes at about 2.6 km. per hour. The term "work" is used loosely.

‡ Important to note that this excess is above the resting "basal" level, and not above the resting level of same experiment. Calculated on the ½ hour period following the beginning of exercise, for reasons given in the text.

TABLE 2

Dog 27 (weight 6.5–7.5 kilos)

DATE	URINE N PER HOUR	RESTING METABOLISM PER HOUR*		INCREASED RESTING METABOLISM OVER "A"	WORK†	EXCESS METABOLISM OF EXERCISE‡			INCREASED EXERCISE METABOLISM OVER "A"
		R.Q.	Cals.			R.Q.	Cals.	Gm. cals. per kgm. of work	
Basal "A"									
1933	*mgm.*			*per cent*	*kgm.*				*per cent*
Jan. 14	76	0.86	11.7		4250	0.84	4.6	1.08	
Jan. 21	69	0.86	11.9		4135	0.82	4.7	1.14	
June 2	46	0.90	11.4		4323	0.82	4.7	1.09	
June 14	31	0.80	11.8		4032	0.84	4.9	1.22	
June 16		0.89	10.9		4027	0.81	4.3	1.12	
June 26	65	0.84	11.4		4754§	0.80	6.2	1.31	
June 27	81	0.87	11.7		4011	0.84	3.8	1.08	
Average		0.86	11.5			0.82		1.14	
Diiodothyronine, January 16, 10.0 mgm. per kilo. Intravenous									
Jan. 17	86	0.89	12.9	8	4283	0.91	5.4	1.23	8
Jan. 19	84	0.83	12.6	6	4250	0.70	5.4	1.19	11
Average		0.86	12.8	7		0.81		1.21	10
Diiodothyronine, January 23, 50 mgm. per kilo. Subcutaneous									
Jan. 24	103	0.80	14.5	22	4077	0.82	6.4	1.57	38
Jan. 25	150	0.84	15.6	31	4068	0.78	6.6	1.62	42
Jan. 26	110	0.85	14.6	23	4082	0.80	5.9	1.45	30
Jan. 27		0.75	15.7	32	4108	0.80	6.6	1.60	43
Jan. 28	84	0.84	14.8	24	4005	0.82	6.9	1.73	54
Average		0.82	15.0	26		0.80		1.59	41
Diiodothyronine, June 5, 50 mgm. per kilo. Intravenous									
June 6	60	0.90	13.1	15	4184	0.81	5.9	1.41	24
June 7	72	0.87	13.6	19	4122	0.90	5.9	1.43	25
June 8	110	0.79	14.5	27	4084	0.84	6.6	1.61	41
June 9	43	0.77	14.8	30	4002	0.73	7.1	1.76	54
June 12	71	0.76	12.7	11	4124	0.79	5.7	1.38	21
June 13	65	0.79	12.2	7	4128	0.75	5.7	1.38	21
Average		0.81	13.5	18		0.80		1.50	31
Thyroxine, June 19, 2.0 mgm. per kilo. Intravenous									
June 20	59	0.85	14.3	25	4044	0.80	6.9	1.69	48
June 21		0.79	13.4	18	4013	0.85	6.8	1.68	47
June 22	85	0.77	13.2	16	4158	0.79	7.0	1.53	34
Average		0.80	13.6	20		0.81		1.63	43

* Average of pre- and post-exercise resting metabolism.

† Running on a horizontal treadmill for 15 minutes at about 2.6 km. per hour. The term "work" is used loosely.

‡ Above the resting "basal" level and not the resting level of same experiment. Calculated on the ½ hour period following the beginning of exercise, for reasons given in the text.

§ The speed of the treadmill was accidentally increased considerably.

figure rose to 1.31 gram cals., and when we had reason to suspect restlessness after the completion of the exercise, there was no noteworthy deviation from this standard of efficiency.

b. *Thyroxine.* This animal showed a weak response to thyroxine,—considerably less than other dogs that we have previously studied (Canzanelli and Rapport, 1933). An intravenous dose of 2 mgm. per kilo produced a total increase of only 200 Cals. above the basal (cf. dog 18). The lower calorigenic action was due chiefly to a decrease in the duration of the response, the average daily increase in metabolism amounting to 20 per cent. As in the case of dog 18, there was in the exercise periods of these experiments after thyroxine, an energy consumption considerably greater than under basal conditions; there was not only summation of the "work" metabolism and the resting calorigenic action, but an excess.

c. *Diiodothyronine.* The response of this animal to diiodothyronine was less than that of dogs we have previously studied, just as it was to thyroxine. An intravenous injection of 10 mgm. per kilo resulted in a very small calorigenic action, as will be seen in table 2. We raised the dose to 50 mgm. per kilo. On giving this amount intravenously, there was a total rise in metabolism of 390 Cals., the average increase being 18 per cent of the basal; the same amount injected subcutaneously resulting in a total rise of 450 Cals., with an average daily increase of 26 per cent above the basal level. We have observed a similar discrepancy between the effects of intravenous and subcutaneous injections in the case of thyroxine, but we have not as yet determined whether it is a constant phenomenon. Upon exercise, there was again the definite difference between the energy consumption of the exercise and recovery period after diiodothyronine and under "basal" conditions, the energy consumption per kgm. of work after the intravenously injected substance being 1.50 gm. cals., and after the subcutaneously injected, 1.59 gm. cals., or 31 and 41 per cent, respectively.

Discussion. The above results show that the resting "waste heat" of the calorigenic action of thyroxine and diiodothyronine cannot be used to perform muscular work. As to thyroxine, this confirms observations made by Plummer and Boothby (1921) on patients suffering from exophthalmic goiter, and it further confirms their observation that in addition to the complete summation of resting calorigenic action and "work" metabolism, there is a considerable excess. Thus, 3 normal persons, in their experiments, required 1.18 gm. cal. per kgm. of work (walking on a horizontal treadmill); whereas 6 exophthalmic goiter patients required 2.28 gm. cals. per kgm., an increase of almost 100 per cent. Since the basal metabolism in these patients was +51 per cent, it appears that not only did this calorigenic action persist during the exercise, but that there was an excess equal to it. This excess is to be observed in our own experiments with thyroxine, and in about the same proportion to the resting calorigenic

effect as in the humans studied by Plummer and Boothby, and we cannot ascribe it to anything but a relative inefficiency in muscular performance, recognizing that this is not explanatory. Kommerell (1931), analyzing similar experiments performed with a varying load on the animal, comes to the mutually contradictory conclusions that thyroxine produces "no change in the efficiency" of work, but that it causes the work to be done "no longer with the same precision." We are unable to follow his reasoning. His animals showed an astonishing lack of efficiency, the normal dogs requiring an energy consumption of 15 to 20 gm. cals. per horizontal kgm. of work. We have never seen a dog that required more than 2.5 gm. cals. per kgm. in this type of work, and they usually, as in the present experiments, require only about 1 gm. cal., as did Plummer and Boothby's normal humans.

As with the thyoxine, so with diiodothyronine, there is not only a complete failure to utilize the extra energy of the calorigenic action for work, but there is a consistent and unmistakable excess which must be labelled a relative "inefficiency." It is of theoretic interest that this excess, common to both diiodothyronine and thyroxine, is not present in the metabolism of exercise after either meat protein or tyrosine (Rapport, 1929), there being in the latter cases only a failure to utilize the energy of specific dynamic action, and no added "inefficiency."

SUMMARY

1. The energy of the calorigenic action of diiodothyronine cannot be used for muscular work. There is, in addition, after giving this substance a relative "inefficiency" in the performance of muscular work compared with the animal under "basal" conditions.

2. The same is true of thyroxine, confirming, in the case of this substance, previous work by Plummer and Boothby.

3. In regard to the second factor—the superimposed "inefficiency," diiodothyronine and thyroxine appear to differ from meat protein and tyrosine.

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THE USE OF ETHYL ALCOHOL AS A FUEL IN MUSCULAR EXERCISE

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In a recent paper Carpenter (1933) has reviewed extensively the literature relating to the effect of muscular exercise on the metabolism of ethyl alcohol, and concludes that it "by no means furnishes adequate data to enable one to draw a decisive conclusion in regard to whether or not muscular activity accelerates the combustion of alcohol." There is no doubt that the evidence is confusing, though some of it can be discounted in view of the inadequacy of the methods employed. In collaboration with Lee and Burdett, Carpenter (1933) has himself recently reported work which indicated that the metabolism of alcohol in the human body is independent of activity. In view of Carpenter's résumé, it is unnecessary for us to review the literature, for which the reader is referred to his paper.

Our interest in the matter derives from previous work in this laboratory, which has led us to the conclusion that the three major foodstuffs,—carbohydrate, protein and fat,—can apparently be used equally well as oxidative fuel in muscular exercise. It would seem, on *a priori* grounds, that alcohol might behave like the other foodstuffs in this respect. On the other hand, it is a fact that certain exothermic reactions of an oxidative character, such as the calorigenic action of protein and certain amino acids, as well as that of hormones like thyroxine and epinephrine, cannot be employed for this purpose. We have therefore performed experiments with ethyl alcohol, to obtain evidence as to the category in which alcohol would fall.

METHODS. Our experimental animal was a bitch weighing about 8 kgm., subsisting on a standard maintenance diet. The respiratory metabolism was obtained by the use of a closed system of the Benedict-Homans type, as previously described in communications from this laboratory. Exercise was performed on a horizontal treadmill within the animal chamber, and consisted of 15 minutes' running at about 2.5 kilometers per hour. Our criterion of the oxidation of alcohol was the respiratory quotient, and the general procedure we used to demonstrate our point was essentially similar to that used by Canzanelli and Rapport (1932). We established the resting post-absorptive R.Q. on a "standard," high carbohydrate, and high fat maintenance diet respectively, and on certain days then exercised the

animal and observed the respiratory quotient of exercise and recovery. On other days, after as before obtaining the resting post-absorptive R.Q., we administered 100 cc. of 10 per cent alcohol by stomach tube, and obtained the respiratory quotient thereafter, which, as we shall show later, demonstrated that alcohol was being oxidized. Then we exercised the dog to see whether or not the quotient would indicate a similar oxidation of alcohol in the excess metabolism of exercise and recovery. This excess metabolism was calculated, as usual, upon the difference between the gaseous exchange of exercise and recovery and that of the average of the pre- and post-exercise resting periods.

The possibility existed that the elimination of alcohol in the breath, particularly if increased during exercise, might introduce an error into the determination of oxygen, which is dependent upon volume changes in the system. We have accordingly performed alcohol checks during which open vessels of alcohol were allowed to vaporize within the apparatus. The amounts vaporized per hour in this manner varied from 10 cc. to 20 cc. Nevertheless, absorption by the acid bottles was sufficiently complete so that no appreciable influence was observed upon the R.Q., the mean, in the alcohol checks, being 0.664, and the maximum deviations being 0.009, of 1.3 per cent. As the maximum elimination of alcohol in the breath of our dog, even during exercise, judging from the experiments of Völtz and Baudrexel (1911), was hardly greater than 0.3 cc. of alcohol vapor, we felt that this source of error could properly be neglected.

EXPERIMENTAL RESULTS. 1. *Experiments with the "standard" diet.* The results of these, as of the other experiments, are summarized in table 1. The "standard" maintenance diet, given at 4:30 in the afternoon, consisted of about 60 per cent carbohydrate, 25 per cent fat, and 15 per cent protein. Three preliminary experiments were performed in which no alcohol was given. In these, the post-absorptive respiratory quotient 16 hours after the last food averaged 0.86 and that of the excess metabolism of exercise and recovery 0.81, the discrepancy of 0.05 between them being rather more than we have usually observed. Following this we performed ten experiments in which alcohol was administered. On the first day (Oct. 23), the effect of the alcohol was not great either on the resting or exercise R.Q., and we have not included it in our calculations. In the remaining nine experiments, the post-absorptive respiratory quotient in the resting state averaged 0.86, with deviations that will be seen in the tables. After removal from the apparatus, and the giving of alcohol, the gaseous exchange was again determined for at least two half-hour periods beginning about three-quarters of an hour after the administration. The average respiratory quotient of these resting periods, and of the resting periods following recovery from exercise, was 0.74, the average figures from the pre- and post-exercise periods respectively happening to be identical. That is,

TABLE 1

DATE	TYPE OF EXPERIMENT	URINARY N PER HR.	RESTING				WORK†	EXCESS METABOLISM OF EXERCISE AND RECOVERY		
			Post-absorptive		After alcohol[1]			R.Q.	Cals.	Gm. cals. per kgm of work†
			R.Q.	Cals.	R.Q.	Cals.				
1933		*mgm.*					*kgm*			
Oct. 19	Post-Absorptive		0.84	11.9			5193	0.79	6.0	1.18
Oct. 20	Post-Absorptive	87	0.88	11.4			4906	0.82	5.3	1.08
Oct. 21	Post-Absorptive	90	0.85	11.8			4935	0.81	4.7	0.95
Oct. 23	10% Alcohol 10 cc.				0.80	11.41	5094	0.82	7.2	(1.41)‡
Oct. 24	10% Alcohol 10 cc.		0.84	11.9	0.75	12.89?	5025	0.88	5.0	0.99
Oct. 25	10% Alcohol 10 cc.	74	0.91	11.8	0.73	12.43	5075	0.95	4.7	0.92
Oct. 26	10% Alcohol 10 cc.	75	0.84	11.9	0.74	12.27	4950	0.86	5.1	1.04
Oct. 27	10% Alcohol 10 cc.	103	0.86	11.6	0.74	11.74	5140	0.88	5.7	1.11
Nov. 6	10% Alcohol 10 cc.		0.82	12.9?	0.76	12.33	4961	0.87	5.0	1.01
Nov. 7	10% Alcohol 10 cc.	79	0.90	11.9	0.74	11.79	5048	0.90	5.8	1.14
Nov. 15	10% Alcohol 10 cc.	68	0.88	12.4	0.74	12.43	5021	0.89	5.5	1.10
Nov. 16	10% Alcohol 10 cc.	78	0.87	11.5	0.75	12.37	5109	0.89	4.9	0.96
Nov. 17	10% Alcohol 10 cc.	71	0.85	11.5	0.75	12.14	5028	0.87	5.4	1.07
Nov. 20	10% Alcohol 10 cc.—High CH Diet	59	0.99	12.4	0.79	12.35	5076	0.92	5.4	1.06
Nov. 21	Post-Absorptive—High CH Diet	63			0.90	11.64	5266	0.97	6.8	1.19
Nov. 22	Alcohol—High CH Diet	75	0.93	12.8	0.78	12.96?	5186	0.97	5.4	1.04
Nov. 23	Post-Absorptive—High CH Diet	49			0.86	12.10	5157	0.90	6.4	1.22
Nov. 24	Alcohol—High CH Diet	56	0.89	11.7	0.75	12.04	5196	1.02	5.6	1.09
Nov. 27	Alcohol—High Fat Diet	83	0.73	12.6	0.69	13.38*	5222	0.87	5.4*	1.04
Nov. 28	Alcohol—High Fat Diet	88	0.70	13.3	0.68	14.12*	5128	0.91	5.5*	1.05
Nov. 29	Alcohol—High Fat Diet		0.72	11.7	0.71	13.15*	5082	0.71	5.2*	1.02
Dec. 5	Alcohol—High Fat Diet	90	0.76	12.4	0.71	12.91*	4955	0.79	5.9*	1.20
Dec. 23	Post-Absorptive—High Fat Diet	53	0.74	13.1			5017	0.72		

* Using factors for fat.

† Used loosely to represent movement of mass horizontally.

‡ Movement in recovery period.

there was a marked fall in the quotient following alcohol when the animal was not active. The respiratory quotients of the excess metabolism of exercise and recovery, however, instead of remaining in the neighborhood of 0.74, reverted practically to the resting quotient before alcohol had been given, the average for nine experiments being 0.89. In no single case did the animal show evidence of oxidizing alcohol for the purposes of muscular exercise, the fuel of exercise being approximately the same mixture of foodstuffs that had been utilized at rest in the post-absorptive state.

2. *Experiments with a high carbohydrate diet.* The maintenance diet in these experiments was about 90 per cent carbohydrate. The procedure was the same as in the first series. The post-absorptive resting R.Q. on the three days when alcohol was given averaged 0.94. In the resting state after alcohol the quotient averaged 0.77, and the quotient (after alcohol) of exercise and recovery averaged 0.97. Here again was indication that the animal was not using alcohol in exercise.

3. *Experiments with a high fat diet.* The maintenance diet was about 90 per cent fat. The post-absorptive quotient averaged, in four alcohol experiments, 0.73. After alcohol the R.Q. consistently fell slightly in the resting state, though naturally not much, the average being 0.70. The quotient of exercise however showed a peculiar phenomenon, being except in one instance not only higher than the resting quotient after alcohol, but considerably higher than the post-absorptive R.Q. as well, the average being 0.82, and in three of the four experiments, 0.86. We are at a loss to account for this. It is apparently not due to the blowing-off of CO_2 as a result of a disturbance of the acid-base equilibrium, for we have followed the metabolism for several hours without seeing any sign of a compensatory effect. It would appear as though, after alcohol, when the animal is on a high fat diet, there were a preferential use of carbohydrate in exercise, but we do not feel at present like drawing any definite conclusion as to this.

4. *Efficiency.* We have calculated the energy requirements per kilogrammeter of "horizontal work," and have noted them in table 1. They show too much variation to permit precise quantitative analysis, and it is sufficient to state that we found no evidence of lowered efficiency when this amount of alcohol was ingested.

Discussion. The fact that energy derived from the oxidation of all three major foodstuffs can be used for muscular work argues for a non-specificity of the oxidative recovery process. From the data given above, it seems clear that ethyl alcohol cannot so be used, and that the energy from its oxidation belongs in this respect in the same category as that of the energy from the calorigenic action of protein, thyroxine, etc. It differs from the latter, however, in that it is not wasted in the resting state, but can be used for the "vegetative" functions, replacing the oxidation of other foodstuffs.

The criterion on which these conclusions are based is the respiratory quotient, and the assumption is made that the quotients obtained are true combustion quotients. Else this paper, as well as countless others, has no validity in respect to the conclusions drawn. In this connection, therefore, we think it timely to say a few words in regard to the respiratory quotient. We regret that space will not permit a discussion in extenso, but we feel it necessary to protest against the denial, sometimes made recently, that the respiratory quotient can be regarded as an index to the nature of the combustions in the body. It is clear that quotients may be affected by the interconversion of foodstuffs, but in our opinion the great weight of evidence at the present time indicates that under all but certain well-defined instances the quotients properly obtained in the intact animal are indeed true combustion quotients. There are two types of criticism of this point of view. In one it seems to us that the wish has often been father to the thought. In arguing that sugar can be oxidized in complete diabetes, or that carbohydrate is the sole oxidative fuel of exercise, or that fatty acids are convertible to sugar, it has been assumed that there are no true combustion quotients, though rather as an hypothesis necessary to the argument than as a conclusion based on direct evidence. In this type of argument curiously enough, the respiratory quotient of unity remains sacrosanct; all other combustion quotients are held invalid at least by implication.

The other type of criticism is based upon doubt as to the mechanism of the production of the respiratory quotient. For example, Thunberg (1930), accepting dehydrogenation, believes that "nothing of the oxygen consumed in the general metabolism is found in the expired carbon dioxide," and that "the oxygen consumed during the respiration is transformed to water." So far as we are aware, Thunberg does not deny the combustion quotient but simply questions its classical explanation, and we mention his theory because it has been used as an argument in favor of the view that combustion quotients are unobtainable. But even if, in the oxidation of sugar, the R.Q. of unity be what one might call a secondary phenomenon, it is certainly not an accidental one. It is no accident that upon forcing the metabolism of given foodstuffs practically theoretical quotients for these foodstuffs can be observed in the intact animal. The situation is in a sense analogous to the law of surface area. That we do not know why the metabolism corresponds to the surface area rather than to the body weight, for instance, need not prevent our acceptance of the fact that roughly speaking it does.

In view of the above, therefore, we feel that with suitable care combustion quotients can be obtained, and as in the present communication deserve to be interpreted as such.

SUMMARY

Ethyl alcohol cannot be used as a source of oxidative energy for muscular exercise, and in this respect differs from the three major foodstuffs.

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THE NATURE OF THE R WAVE POTENTIALS IN THE TORTOISE AND FROG HEART

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The purpose of the experiments to be described in this communication is an attempt to determine the fundamental nature of the electrical change in the ventricular muscle during the period of the QRS group of electrocardiographic potentials. The method is to record the potentials from the heart in a conducting field of a form in which the theoretical distribution of potentials due to charges is known and to compare this theoretical with the actual distribution determined from hearts in such a field.

Action potentials have been in most cases explained on the "negativity hypothesis," which postulates a fall of potential on the surface of the active region, progressing in the form of a wave accompanying the spreading activity. Certain observations, both experimental and theoretical, are not in accord with this conception. Craib, in an important series of studies on heart and skeletal muscle, has shown that in order to explain the curves actually obtained, it is necessary to postulate a rise of potential above the resting level as well as a fall below this level. The "simultaneous presence of an anode and a cathode situated at the tissue surface and arising from a common electromotive force within the tissue," is defined as a doublet or dipole in this connection (Craib, c). During the QRS potentials, there was evidence that the dipoles responsible were of relatively short duration and progressed with the positive charge or anode in front of the negative charge or cathode. Craib attempted to substantiate this hypothesis by comparison of the R potentials from a tortoise heart with those from an artificial dipole placed within a volume conductor of a form in which potential distribution was known theoretically (Craib, a). He chose a spherical conductor, consisting of a glass globe filled with Ringer's solution. The heart or artificial dipole, the latter consisting of the two poles of a battery close together, was placed at the center of the sphere and the fall of potential determined along some axis, as the distance from the center was increased. The potentials fall rapidly under these circumstances and when the tortoise heart is the source, assume a very low value at moderate

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distances from the heart. Craib used an unamplified string galvanometer for recording, and the small size of the movements obtained make questionable the accuracy of measurement. He, however, found evidence of agreement between the rate of fall of potential with the heart and with the artificial dipole as the source, and both approximated that deduced theoretically.[2]

Wilson, Macleod and Barker (a, b), as a result of both experimental and theoretical studies, have come to essentially the same conclusion as to the nature of the fundamental electrical situation responsible for action potentials. They connected a point on the exposed auricle of the dog's heart in situ through a string galvanometer to a lead from the hind leg. The initial movement of the auricular portion of the electrogram thus recorded was in the direction indicating a rise of potential under the auricular electrode. This was succeeded by a fall of potential and a final return to the resting position. On moving the auricular electrode nearer the point of origin of the cardiac impulse, namely, the sino-auricular node, the initial positive potential became less and disappeared when the electrode was placed directly over the node. In the latter case the curve was monophasic or nearly so, showing a fall of potential. Interpretation of these results led, in agreement with Craib, to the assumption of a moving dipole, with positive charge preceding. At the origin of this dipole at the sino-auricular node, the electrode is affected only by the receding negative charge and indicates only a fall of potential. At points away from the node, the electrode is first affected by the approaching positive charge and later by the receding negative charge of the dipole. The potential curve is thus diphasic with an initial rise. Wilson, Macleod and Barker (c) also carried out an extensive mathematical analysis of the distribution of potentials due to dipoles in volume conductors of various kinds. The theory of origin of the electrical change in active tissue that has had the widest consideration and the most extensive experimental support is the membrane theory of Bernstein. According to this theory there exists on membrane interfaces of resting cells a state of "ionic polarization," arising from the impermeability of the membrane to anions. The result is an excess of positively charged ions on the outer surface of the cell, an equilibrium being established between diffusion pressure and electrostatic attraction and

[2] The potential distribution in a spherical conductor with a dipole at the center is given by $V = K \cos\theta \left(\frac{1}{r^2} + \frac{2r}{R^3}\right)$ where K is a constant, θ is the angle that the line connecting the point at which the potential is measured and the center of the dipole makes with the axis of the dipole, r is the distance of this point from the center of the dipole, and R is the radius of the sphere. The constant K is determined by several factors; the distance apart of the poles of the dipole, the quantity of electricity entering per unit time, and the specific conductivity.

repulsion.[3] This selective permeability disappears during activity, and the membrane becomes depolarized. By the application of the laws of potential theory, Wilson, Macleod and Barker (c) showed that the advancing wave of depolarization could be replaced theoretically by a moving charged membrane, the positive charges being located on the face toward the direction of movement. The imaginary membrane is placed at the junction of the polarized and depolarized regions and is supposed to progress at the same rate as the depolarization process. At a distance from the heart, or on the surface of a thin sheet of muscle, such as the mammalian auricle, it is equivalent to a dipole or series of dipoles with positive charges oriented in the direction of the advancing depolarization.

In the experiment of Wilson, Macleod and Barker on the dog's auricle and in many of the experiments of Craib, the leads to the galvanometer were from the muscle and from some distant point in the conducting field with which the muscle is in contact. In Craib's work, the muscle was placed on a filter paper pad wet in Ringer's solution, and the distal lead made from the edge of the pad as far as possible from the muscle. Potentials recorded by such leads are regarded as representing the potential changes at the proximal electrode on the muscle. The distal electrode is so far away in the field from the source of potential, that the potential changes under it are regarded as insignificant and thus contribute to the recorded potential only to a negligible extent. On passing away from a source of potential in a volume or plane conducting field, the potential falls at first rapidly then more slowly. Along any line the curve is of hyperbolic form and the potential at distant points is only a small fraction of the potential near the source. The question has been investigated on theoretical grounds by Wilson, Macleod and Barker (d) in reference to the comparative potential changes at an electrode placed directly on the exposed dog's heart and at a lead from a limb. Using the Einthoven equilateral triangle as a basis of analysis, it was shown that the potentials at the apices of the triangle can be only a small fraction of the potential at the heart.[4]

[3] The electromotive force across the membrane follows the law of the concentration cell and is given by the Nernst formula, $E = \frac{RT}{nF} \log_e \frac{C_1}{C_2}$ where R is the gas constant, T the absolute temperature, F the Faraday equivalent, n the number of diffusible cations and C_1 and C_2 the concentration of the cations outside and inside the membrane respectively.

[4] The potential at the lower apex, corresponding to the lower extremity in leads II or III is given by $\frac{E_2 + E_3}{3}$ where E_2 and E_3 are the potentials recorded in leads II and III of the standard electrocardiographic leads. Since E_2 and E_3 are components of the actual potential at the heart, the value of the expression is evidently small. In one lead directly from the dog's auricle, the other lead from the extremity, the corrections in potential due to the distal lead amounts to approximately two per cent of the recorded potential.

The view that the potential changes at the distal electrode are so small as to be negligible has been attacked by Bishop and Gilson (1927, 1929). In leads from the heart and a distal portion of the body, the distal electrode, since it is in electrical connection through the conductive tissues of the body with the heart, is regarded as being affected, especially as to polarity, by the heart. Since the potential measurements in the present work were based on the assumption of negligible potential changes at the distal electrode, we have also considered it important to investigate this matter further, particularly under the conditions of our experiment.

METHODS. A plate glass disc, provided with a rim 43 cm. in radius, was mounted on a turntable with ball bearings. The disc was marked with lines radiating from the center and forming plane angles of fifteen degrees between each line. There were thus six radial lines in each quadrant or twenty-four for the entire circle. Two non-polarizable electrodes of the zinc-zinc sulphate type were placed at opposite sides of the edge of the disc. A bridge extending the full diameter of the disc was provided with a sliding plate carrying a third electrode. The disc was carefully levelled and Ringer's solution poured on it to a depth of 3 mm. The heart was mounted inside a porous ring 5 cm. in diameter, which was laid on the disc with the center of the preparation at the center of the disc. The ring extended above the surface of the Ringer's solution and prevented the propagation of liquid waves produced in the Ringer's solution by the contraction of the heart from passing outward and affecting the potential of the outlying electrodes. The preparations were mounted in all cases for approximate isometric contraction, but waves arising in the immediately surrounding liquid could not be entirely prevented.

The two oppositely placed electrodes at the edge of the disc were connected together and to the grounded side of the input of a two-stage direct current amplifier. This amplifier has been described in a preceding publication (Eyster, Maresh and Krasno, 1933). It serves two purposes, first to provide a high resistance input and thus to allow potential rather than current flow measurements to be made; and second, to supply sufficient sensitivity to record the low potentials present at all but the nearer points in the field. The movable electrode or exploring electrode was connected to the grid side of the amplifier input. The output from the amplifier was connected to a string galvanometer. The experiments were carried out in a room with walls, floor and ceiling shielded with metal and grounded. The single electrical circuit entering from the outside, that from a direct current generator for the arcs and the motor of the photographic registration apparatus, was shielded and trapped to ground for electrical strays. With these precautions, satisfactory records could be obtained with a sensitivity approaching the "noise level" of the first amplifier tube of the order of 1 microvolt or less. The galvanometer string was adjusted to normal

tension and the desired sensitivity obtained by varying the extent of amplification. The galvanometer was so connected that relative negativity of the movable electrode caused an upstroke on the record.

Preliminary experimentation with the isolated heart made it obvious that the potential curve recorded along different axes had different time relations; in other words, the curves were functions not only of space but of time. Along any single axis the curves are the same except for total potential values. Along different axes at the same instant of cardiac activity the peaks or other points, however, do not correspond. It was necessary to have some reference curve from which identical points in the activity phase could be obtained. For this purpose two additional non-polarizable electrodes were placed in contact with the base and apex respectively of the ventricle, leading directly to a second string galvanometer recording simultaneously on the same record. The electrogram thus obtained was constant and independent of the position of the exploring electrode. Usually the sharp peak of the R wave in the curve obtained by direct leads from the heart was used as the reference point in measuring potentials on the simultaneously recorded potential curve.

EXPERIMENTAL PROCEDURE. All vessels entering and leaving the heart were ligated, and the organ filled with blood removed and mounted within the porcelain ring as described. The preparation was placed at the center of the disc with the axis of minimal potential change corresponding with the axis of the indifferent electrodes. Wick electrodes attached to the base and apex of the ventricle led to one of the galvanometers. The exploring electrode was now placed at a certain fixed distance from the heart along some axis and connected through the amplifier to the indifferent electrodes. A record of deflections in the two galvanometers was obtained. The table was then rotated through an angle of 15 degrees to bring the exploring electrode on a new axis and a second record was made. This was repeated until the exploring electrode made a complete circle around the heart, comprising twenty-five records in all. The exploring electrode was now moved to a new distance from the heart and the procedure repeated. In some cases a third series of records at a third distance was made. The method provided for the rapid taking of the numerous records at a time when the preparation was in good condition. Three complete rings, comprising 75 records, can be obtained in less than an hour. Calibration records were made after each sixth potential record. The electrogram, obtained by the leads direct from the heart, served two purposes, first to determine whether or not the potential fell during the course of the experiment at its source, the heart, and to allow corrections to be made if necessary; and second, to provide a time reference point for the potential curves. All records were made on bromide paper, 6 cm. in width, and at a speed of 15 cm. per second.

Critique of method. This method for plotting the potential field around the heart is based on the assumption that the potential changes at the distant or indifferent electrodes are insignificant, and that the potential actually recorded during the heart beat is the potential due to the heart at the position of the proximal or exploring electrode. In the case of a single stationary dipole such as that produced by connecting two electrodes close together at the center of the field to a battery, this can be insured by placing the indifferent electrode along the axis of zero potential, namely, that axis at right angles to the axis of the dipole. It develops, however, as will be seen, that in the case of the heart, due to the fact that the situation is more complex than that of a single dipole, there is no axis along which throughout the course of the R complex the potential is zero. At any instant there is a zero axis, but its position shifts during the period of the potential change. There is, however, an axis of minimal potential change, nearly, but not quite coinciding with the anatomical transverse axis of the ventricle, and the two indifferent electrodes were always placed at the extremities of this axis.

On passing out along any axis away from the heart, the potential falls in the form of a hyperbolic curve, at first very rapidly and then with progressively decreasing rate to become asymptotic to the abscissa. At the margin of the disc the actual measurement of potential gives values of less than two microvolts. The curve of potential change with increasing r for a field as large as the one we have employed would clearly point to insignificant potential changes at the margin, as compared with those within 20 cm. of the heart. Due to the opposing views in reference to this matter it was deemed necessary, however, to test it directly under the conditions of our experimental procedure. The following experiment was performed. A tortoise heart was mounted at the center of the disc and the indifferent and exploring electrode connected in the usual manner through the amplifier to the string galvanometer. Instead, however, of moving the exploring electrode to various positions as in the usual procedure, it was kept constant at 10 cm. distance from the apex (270° axis), and the indifferent electrodes were moved along the margin of the disc at the extremities of the various axes, records being made along each axis. The exploring electrode was now moved out to 20 cm. distance and a second set of records obtained. The different curves obtained were of identical form and varied only slightly in potential. The curves were diphasic showing an initial rise of potential at the proximal electrode, followed by a smaller negative phase. At the 10 cm. distance the initial positive potential varied between 26.8 and 24.4 microvolts in the different positions of the distal electrodes. At the 20 cm. distance the corresponding range was from 14.1 to 13.5 microvolts. The final negative phase was less than half this magnitude and showed even less variation. The recording equipment was used at a

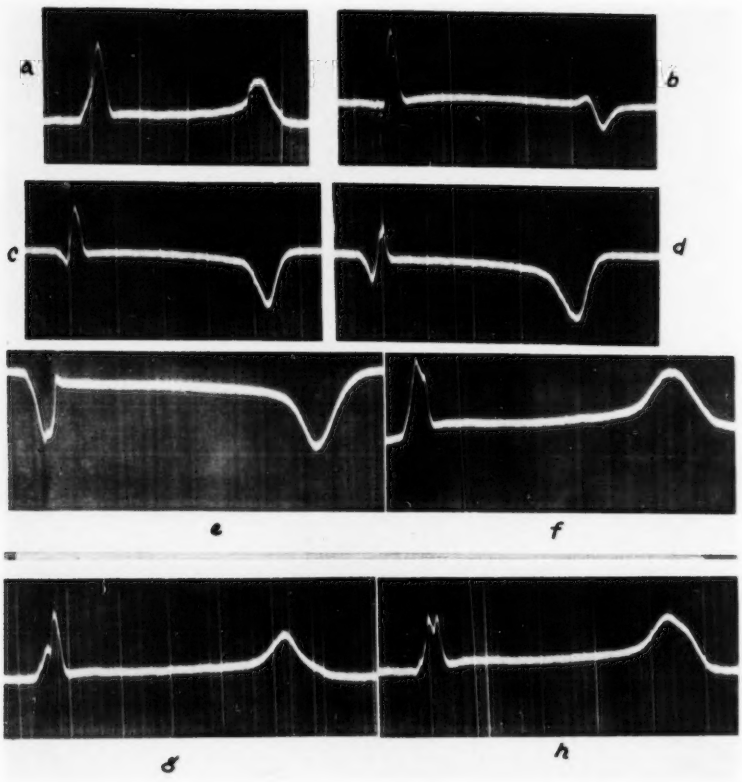

Fig. 1. Excised tortoise heart fixed in circular porcelain ring and placed at the center of a circular disc conducting field 43 cm. in radius and approximately 1 cm. thick. The heart was completely immersed in the conducting medium (Ringer's solution). Leads to amplifier from indifferent electrodes at the edge of disc along axis of minimal potential and from an exploring electrode placed at various points on the ventricular surface or in the conducting medium a short distance from the heart. An upward movement of the curve indicates a fall of potential at this electrode. The vertical lines record time intervals of 0.04 second. (About one-fourth original size.)

a. Lead from ventricular base near origin of great vessels. The R wave shows a fall of potential of 1.81 mvs.

b. Lead from a region on longitudinal axis of ventricle, on anterior surface and on the cross axis midway between base and apex. The R wave shows a fall of potential of 2.24 mvs.

c. Lead from a region on longitudinal axis of ventricle, on anterior surface and between mid ventricle and apex. The R complex shows an initial rise of potential of 0.58 mvs. followed by a fall of potential of 2.1 mvs.

sensitivity of 0.46 and 0.85 mm. per microvolt in the two sets of records. It may therefore be concluded that as far out as 20 cm. the potential of the exploring electrode is affected to only a small extent by the relative position of the indifferent electrodes, at the margin of the disc and 43 cm. away from the heart. The difference approximates that of the error of experimental measurement and may be ignored.

As has been stated previously, the results of Craib and of Wilson, Macleod and Barker, have been criticized by Bishop and Gilson on the basis that the potential of the so-called indifferent or distal lead may be affected by return circuits through the tissues or conducting field to parts of the skeletal muscle or heart lying in contact with the field. To still further test this criticism, we have carried out experiments similar to those of Craib and of Wilson, Macleod and Barker, but in which the heart was completely immersed in the conducting medium, thereby eliminating possible preferential paths of conduction to isolated regions. The usual technique was employed with the distal lead at the edge of the large disc, but the exploring electrode was placed on the ventricular surface or in regions of the medium close to the heart. Records from an experiment of this type are given in figure 1. Points along the longitudinal axis of the ventricle above a transverse axis approximately midway between apex and base show monophasic R potentials in a negative direction. The extent of potential fall increases at first as this line is approached. Below this line the curves are diphasic with an initial positive potential. As the apex is approached the initial positive potential increases, the succeeding negative phase decreases. In the conducting medium just beyond the apex, the R potential is monophasic and in a positive direction. There may occur a very minute terminal negative change. Placing the distal electrodes along different axes at the margin of the disc did not affect appreciably the magnitude or form of the curves obtained.

EXPERIMENTAL RESULTS. The data obtained by the application of the method just described may be considered from several different standpoints. The potential along any single axis at different distances from the heart may be plotted. In this instance, since the potential curves have

d. Lead from anterior surface of ventricle near apex. The initial rise of potential of the R complex is 0.59 mvs., the succeeding fall 0.825 mvs.

e. Lead from the field 5 mms. beyond apex. The initial rise of potential of the R complex is 0.6 mv.

f. Lead from the field 13 mms. beyond ventricular base. The fall of potential of the R complex is 0.425 mvs.

g. Lead from the ventricular base near left border. The first peak on the R complex corresponds to a fall of potential of 0.55 mvs., the second to a fall of potential of 1.2 mvs.

h. Lead from ventricular base near right border. The maximum fall of potential during the R complex is 0.715 mvs.

the same time relations, the second galvanometer record direct from the heart is unnecessary. The form of these curves and their relation to the shape of the conducting field have been considered in a previous communication (Eyster, Maresh and Krasno, 1933). They are of hyperbolic form, the potential falling at first very rapidly and then more slowly. From a series of such curves obtained along all the different axes, points of equal potential may be selected and plotted on appropriate coördinate paper as equipotential points. Continuous lines joining equipotential points constitute a field of equipotential lines. Along any single line in such a field the potential is the same, on passing from one line to another, there is a potential change or gradient. The individual hyperbolic curves used for this must, however, represent synchronous potentials in the different axes; the use of a second galvanometer with reference curve direct from the heart is necessary, some point on this curve, usually the R peak being selected and the potential on the other curve measured at this instant. Another method of representing the potential distribution is to plot the actual potentials measured at various angles but at constant distance from the heart or dipole. Theoretically, in a circular disc field, this should give a series of circles passing through the origin.[5] That this disposition is obtained with a single dipole under the conditions of our experiment is shown in figure 2. The large circles represent the theoretical distribution, the small plotted circles the values obtained experimentally by placing an artificial dipole at the center of a disc conductor. In figure 3, equipotential lines as determined from a similar artificial dipole are drawn. The figure accords so closely with the theoretical[6] as to be indistinguishable, and hence the latter is not reproduced. Finally, the form and time relations of the potential curves obtained along the different axes may be studied.

It has been generally assumed that, at any given instant, the potential developed at the heart may be adequately represented by a single potential source and sink (dipole) of a certain direction and magnitude, provided that the distance from the heart is relatively great as compared with the size of the organ. This assumption forms the basis for the classical analysis of the electrocardiogram by Einthoven, Fahr and De Waart. If this is true, the potentials from the heart at the center of a circular disc conductor should show the same distribution as those represented in figures 2 and 3. When the potentials at some chosen instant during the development of

[5] The potential distribution due to a dipole at the center of a circular homogeneous disc of radius R, is given by $V = K \cos\theta\left(\frac{1}{r} + \frac{r}{R^2}\right)$, where θ and r have the same meaning as in the similar equation for the sphere, and the constant K includes in addition to the factors named, the thickness of the disc. With r as a parameter, the expression reduces to $V = K_1 \cos\theta$, the equation in polar coördinates of circles passing through the origin and symmetrical with respect to the polar axis.

[6] The values obtained by regarding V as the parameter in the above equation.

the QRS group in the tortoise or frog heart are plotted in either of the two ways described, it is found that while in general the distribution resembles that due to a dipole, there is a definite departure, as figures 4 and 5 show. Considerable dissymmetry is apparent, and the potentials for a constant value of distance from the heart are greater in general around the base than the apex. The distribution is also not symmetrical with respect to the anatomical axis of the heart. The line of zero potential is bent. On plotting synchronous potentials the potential along some one axis reaches zero, but there is no axis related to the heart which shows zero potential throughout the course of the R complex, thus differing fundamentally from the case of a single dipole.

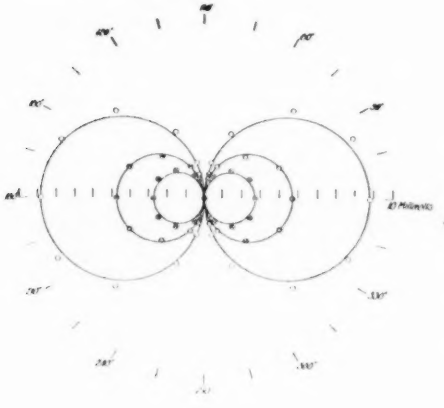

Fig. 2

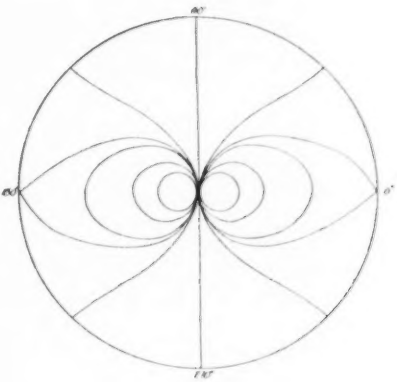

Fig. 3

Fig. 2. Dipole 1 cm. apart in a plane circular conductor 25 cm. in radius 25 ~ A. C. Lines: from the expression $V = A \cos\theta\left(\frac{1}{r} + \frac{r}{R^2}\right)$. Points are determined experimentally, where ⊗ r = 20 cm., Θ r = 10 cm., ○ r = 5 cm.

Fig. 3. Equipotential lines around a dipole with each pole 10 mm. from the center of a circular plane conductor and on the 90–270° axis. 1000 ~ A.C.

These facts indicate that, in the case of the heart in a large conducting field of circular form, the source of the potential is not as simple as has been assumed; and that even when the distance at which the potential is measured is large as compared with the size of the heart it cannot be referred to as an equivalent single dipole, or an electrical source and sink within the organ.[7] A somewhat similar distortion of the field occurs when a purely

[7] It has been suggested to us by Dr. F. R. Wilson that the distortion may be due to the heart disturbing the homogeneity of the conducting field. The potential field around the frog's gastrocnemius muscle is however much less distorted and approximates, within the errors of measurement, that due to linearly directed dipoles. Details of this will be given in a subsequent communication.

physical arrangement is studied in which the single source and sink is replaced by a set of three electrodes at the center as shown in figure 6.

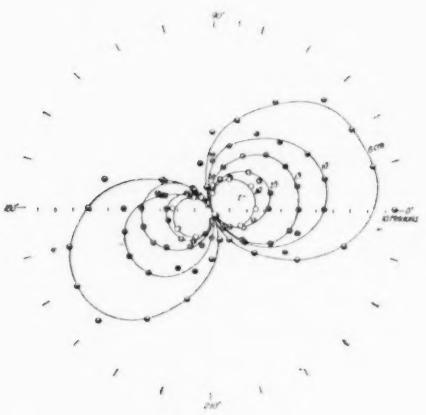

Fig. 4

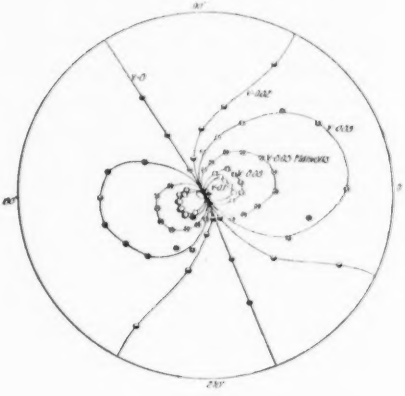

Fig. 5

Fig. 4. Synchronous R potentials from a terrapin heart on a circular plane 43 cm. in radius.

Fig. 5. Equipotential lines for synchronous R potentials around a terrapin heart in a plane circular conductor 43 cm. in radius.

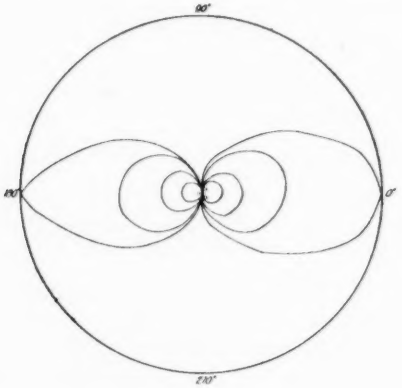

Fig. 6. Equipotential lines around a double and one single pole in a circular plane of 43 cm. radius 1000 ~ A.C. (headphones used). Three electrodes 20 mm. apart.

Examination of the form of the potential curves and their relation to a constant time reference point, when recorded along the different axes,

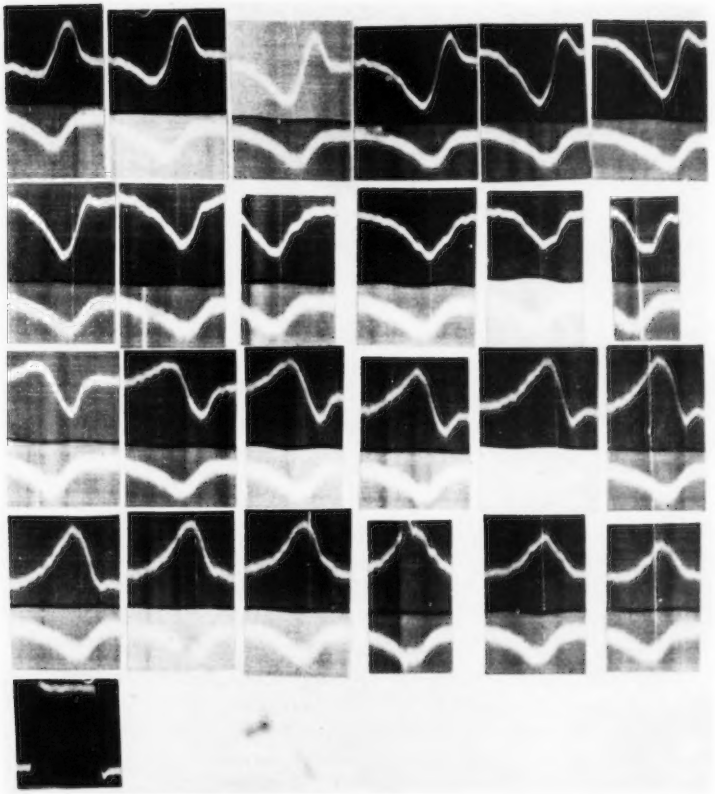

Fig. 7. Isolated tortoise heart placed in the center of a glass disc of 4.3 cm. radius containing Ringer's solution to a depth of three millimeters. In each record the curves show the deflection of the strings of two galvanometers recording simultaneously. The upper curve is the potential during the period of the R complex along various axes at a constant distance of 1.5 cm. from the center of the heart. The exploring electrode was placed on the axes at this distance. The indifferent electrodes were placed at the ends of the 165° and 345° axis and connected together. The long axis of the heart was on the 90°–270° axis, the apex on the 90° the base on the 270° axis. An upstroke on this curve indicates a fall of potential at the exploring electrode. The leads were connected to the galvanometer through a two stage direct coupled amplifier, giving a sensitivity of 0.52 mm. per microvolt. The last record is the response of this galvanometer to a difference of potential at the amplifier input of 50 microvolts. The lower curve is an electrogram obtained by direct leads from the apex and base of the ventricle. The downstroke represents relative negativity of the base. This curve is constant in all records and furnishes a time reference point. The first row of records includes the axes 0° to 75°, the second 90° to 165°, the third 180° to 255° and the fourth 270° to 345°, reading from left to right in each row. The speed of recording was 1.5 cm. per second in all records. (About one-fourth original size.)

shows clearly the complexity of the potential source in the cardiac muscle. Curves from a typical experiment are given in figure 7 and a series of similar curves traced from the original and placed in reference to the axis along which they were recorded in figure 8. The continuous line passing through these curves represents a fixed point in the electrogram recorded by leads direct from the heart. The arrows follow the various wave peaks. Examination of the curves shows a progressive change in wave form from mono-

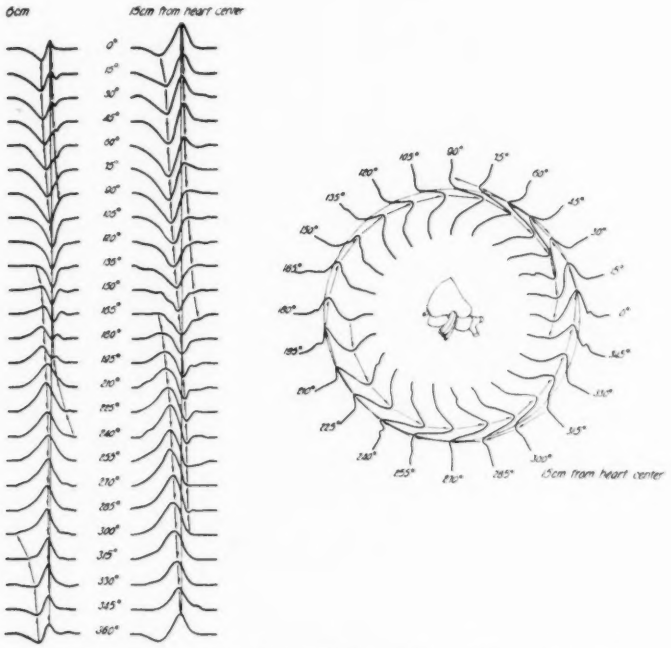

Fig. 8. Changes in the R complex with the angle. The continuous line marks synchronous potentials. Arrows indicate time shift of maxima. Terrapin heart in a plane circular conductor, 43 cm. radius.

phasic to diphasic and the reverse. The time relations of the wave crests show also progressive changes. The curves obtained near the long axis of the heart are monophasic, those near the cross axis, diphasic. On opposite sides of the heart they are in every case symmetrical mirror images of one another. Those in the basal direction show a fall of potential, those in the apical direction a rise of potential. To the left of the heart the potential at first falls and then rises, to the right it first rises and then falls. Numerous experiments of the type recorded in figure 8 have been carried out, on

the heart of the frog as well as the tortoise, and the relations described are found as constant features.

Discussion. It is apparent on theoretical grounds that the potentials recorded from points close to or at a distance from active muscle or nerve will depend upon the volume conductor surrounding the tissue. The shape of this conductor, its extent and its homogeneity or heterogeneity, will all have an effect. Craib (b) has shown experimentally that the form of the potential curve and its polarity relations, as well as its magnitude, depend on the nature of the conducting field surrounding it. A large part of the experimental work on action currents or action potentials has been carried out on muscle or nerve suspended in air or in situ in the body. In the latter case, particularly when one or both leads are direct from the active tissue, the conditions would appear extremely complicated and the accurate interpretation of results impossible. Many electrical circuits, offering paths of varying resistance, present in a field of undefined contour and extent, comprise an extremely complex situation impossible with our present knowledge to interpret theoretically and hence to gain an insight into the fundamental process responsible for the action potential curves obtained. The line of experimental attack in this problem most likely to yield accurate information as to the fundamental electrical changes responsible for the action potential curve would obviously insure two things, first that the active tissue be immersed in a homogeneous volume conductor of known contour and in which potential distribution can be theoretically formulated; and second that the potential field around the active tissue be determined accurately and examined in the light of potential theory. This we have attempted to do in the case of the R potential complex of the spontaneously beating tortoise and frog heart. These hearts were selected because of their long survival period after isolation, and their small size in relation to the conducting field in which they were studied.

It would appear evident that the assumption of a simple lowering of potential, as determined by the theory of "negativity" is insufficient to account for the results obtained in these experiments. Curves such as *e* of figure 1 clearly demand, for their interpretation, a rise of potential above the resting level. It should be recognized that this is by no means in antagonism with the membrane theory, in reference to which the negativity hypothesis is usually explained, but only the proper interpretation of this theory. The membrane theory, as is clear from the analysis of Wilson, Macleod and Barker (c), provides for such a rise of potential when interpreted in the light of potential theory.

The experimental results described indicate clearly that the potential distribution during the R complex in the tortoise and frog's heart has as its basis the development of dipoles. The situation however is somewhat

more complex than that of an equivalent dipole, which means the effectual summation of all dipoles present in the heart into an electrical force which produces the same distribution of potential at a relatively distant point as would be produced by a single dipole situated at the position of the heart. The inference is that we are dealing with more than one equivalent dipole, differing in orientation and probably in time of development. In this connection it is of interest to determine, by detailed consideration of the types of potential curves obtained at different points in the field, the simplest configuration of dipoles which would explain the recorded curves, at least to a fair approximation. The simplest configuration of sources and sinks which can account qualitatively and quantitatively for the potential distribution in space and time, appears to be that represented in figure

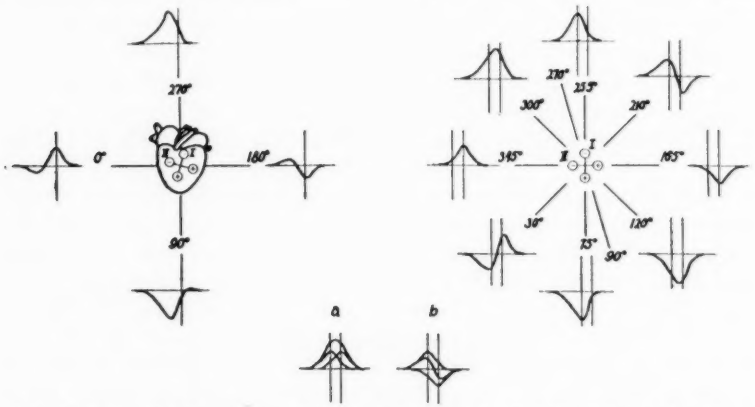

Fig. 9. Suggested disposition of dipoles to account for the distribution of potential around the heart.

9. This disposition was first suggested by a consideration of the four experimental curves at the left of the figure. Assume that dipole I develops first, reaching its maximum potential at the instant given by the first time reference line of the right hand set of curves. At a later instant, dipole II develops, reaching its maximum at the instant given by the second time reference line of the same figure. At any point distant from the heart, possible motion of the dipoles within the confines of the heart would have little effect on the form of the curve and we may consider them as at rest and merely developing and decaying in the manner described. Consider first the influence of the first dipole along the 255° axis. Its growth and decay would result in a lowering of the potential followed by a return to its original potential. The result along this axis would be a monophasic negative variation, the maximum of which will be coincident with the first

time reference line. Along the opposite or 75° axis, the potential variation will be precisely the same in time and wave form, but will be opposite, or positive. Similar negative and positive monophasic potential variations due to dipole II will occur on the 345° and 165° axes respectively, but the maximum of these changes will fall on the second time reference line.

Dipole II will have no effect on the potentials along the 225° or 75° axes, nor will dipole I affect the potential along the 345° and 165° axes, since their components in these directions are zero. On all other axes but those corresponding to the direction of the two dipoles, however, the resulting potentials will be due to the sum of the effects of the two dipoles. Thus on the 300° axis, the effect of dipole I, which is determined by its projection on this axis, will be that of a negative variation the maximum of which will be on the first time line. The effect of dipole II, determined by its projection on the same axis, will also be a negative variation, the maximum of which falls on the second time line. The potential variation on this axis should be the sum of these two monophasic changes. This sum, as shown in figure 9a, gives a curve with a broad top, the maximum of which falls midway between the two time reference lines. The curve on the 120° axis will be similar in form and magnitude but in the opposite direction, indicating a monophasic positive potential change. Finally, the curve on the 210° axis, due to the components of the two dipoles, will be diphasic. The component of the first dipole will cause a negative, the component of the second dipole a positive potential variation. These will sum to produce the curve shown in figure 9b. The curve on the 30° axis will be a reflection of that on the 210° axis. It should be noted that the peaks of the negative and positive variations do not fall on the two time reference lines, but occur before and after them respectively. The effect of the second dipole comes in before the potential maximum due to the first dipole is reached, resulting in the curve turning downward before the first time reference line. The potential minimum due to the second dipole is similarly influenced by the first dipole.

Detailed examination of curves of the type reproduced in figure 8 shows that the assumption of two summated dipoles as described above explains at least the major features of the potential changes observed. Certain minor variations in many curves, particularly those recorded close to the heart, suggest the necessity of considering a third summated dipole of minor importance.

CONCLUSIONS

The potential distribution in a circular disc conducting field of large dimensions surrounding the tortoise and frog's heart during the period of QRS complex resembles that due to a dipole but it is somewhat more complicated. It may be approximated by the combination of two dipoles, oriented in the general direction of base to apex with the negative charge

at the base, the positive at the apex, developing asynchronously. The potential curves recorded in the outlying field are functions of space and time, and demand for their interpretation a rise of potential above as well as a fall below the level of resting potential. This interpretation is in accord with the membrane theory of action potentials, but is opposed to the usual interpretation of the classical negativity hypothesis.

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EXPERIMENTAL DIABETES INSIPIDUS: ITS RELATION TO THE ANTERIOR AND POSTERIOR LOBES OF THE HYPOPHYSIS

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Three different theories have been held in recent years regarding the etiology of diabetes insipidus. Some workers think that the syndrome is due to a disturbance in either the posterior lobe or the intermediate lobe of the pituitary gland; others believe it to be caused by a lesion in a water center in the hypothalamus; and still others, combining the first two theories, postulate a lesion in a nervous pathway between the hypothalamic centers and the posterior hypophysis (Greving, 1926; Pines, 1927). Inasmuch as these theories have recently been discussed very fully by Futcher (1931), Cushing (1932) and Staemmler (1932), it will not be necessary to review them here. It may be noted in passing, however, that with one or two exceptions all of the workers have failed to consider the anterior lobe as an etiological factor in the disease.

The present experiments demonstrate a fundamental rôle on the part of the posterior hypophysis, and at the same time show definitely that the anterior lobe also is very important. These experiments, which are a continuation of previous work on experimental diabetes insipidus (Richter, 1930), demonstrate the effects produced on water-intake in rats by partial and total removal of the pituitary gland.

Effects produced on the spontaneous activity by hypophyseal extirpation have been discussed in a previous paper (Richter and Wislocki, 1930). Since that paper gives only a cursory treatment of the changes in water-intake, the data have been reëxamined and the results presented below together with those from a recent and more extensive investigation.

Inasmuch as it was not possible to be sure of a separation of the intermediate and posterior lobes the term posterior lobe as used herein includes the intermediate lobe as well.

METHODS. Sixty-six rats were used in these experiments, of ages varying from 34 to 227 days.

The animals were kept separately in activity cages consisting of a revolving drum with a cyclometer, and a living compartment with a food-cup

and water bottle (Richter, 1927). Daily records were made of the running activity, the food-intake, the water-intake, and vaginal smears. Body weight was recorded once weekly. The rats were placed in the activity cages at the usual age of 35 to 55 days but no operations were performed until a base line of water-intake was established.

The technique of total and partial hypophysectomy (described in a previous paper) was essentially the same as that used by Smith (1927) except that the sphenoid bone was approached from behind the nasal cavity rather than through it, and a dental drill was used in preference to the hand trephine. The gland was removed by suction, the anterior lobe sometimes totally, sometimes only partially; the posterior lobe, because of its position and location was usually completely withdrawn even when a large piece of the anterior lobe remained.

Autopsies were performed to determine how much pituitary tissue remained. The rats were killed, india ink having been injected through the heart, and the brains were removed with the lepto-meninges intact over the hypophyses. At the same time a note was made as to whether the stalk was intact or severed, and whether the underlying brain stem was injured or not. The brain and all glands of internal secretion were preserved for histological study.

A check on a diagnosis of total or partial removal of the pituitary gland is furnished by the food-intake and body-weight records. With total removal the food-intake shows a decrease as great as fifty per cent in some instances, and the body-weight drops off very sharply, whereas after partial removal such changes do not occur. These two features are quite as reliable as the autopsy findings, and in some instances even more so. Vaginal smears offer another criterion because complete hypophysectomy eliminates the four-day oestrous cycles.

Results. *Complete removal.* It was found that complete removal of the hypophysis in thirty-four animals produced a decrease in water-intake in six, and typical diabetes insipidus in twenty-eight. In no instance was the diabetes insipidus permanent, however, and after several weeks oliguria developed even in the animals which had shown polydipsia and polyuria. The two types of curves are presented in figure 1 with the water-intake in cubic centimeters given on the ordinates and time in days on the abscissae. It will be noted that in the first animal (fig. 1A) the hypophysectomy produced an increase in water-intake from a level of 50 cc. to a peak of 144 cc. per day. This rise lasted only 10 days, however, and within 15 days postoperatively the curve had fallen below the preoperative average. Figure 1B shows only a decrease in water-intake. In both animals a permanent sharp drop in food-intake occurred.

The average daily water-intake for the thirty-four animals decreased from 31.8 cc. for the ten days immediately before the hypophysectomy

to 22.2 cc. for the last ten days of life, a decrease of 29 per cent. The highest water-intake recorded for any one day after the operation was 158 cc. while the average for the group was 68.0 cc. The food-intake decreased from 14.1 grams for the ten days preceding the operation to 6.2 grams for the last ten days of life.

It must be stated that this group includes one of the ten animals classified in the previous paper by Richter and Wislocki as having had a

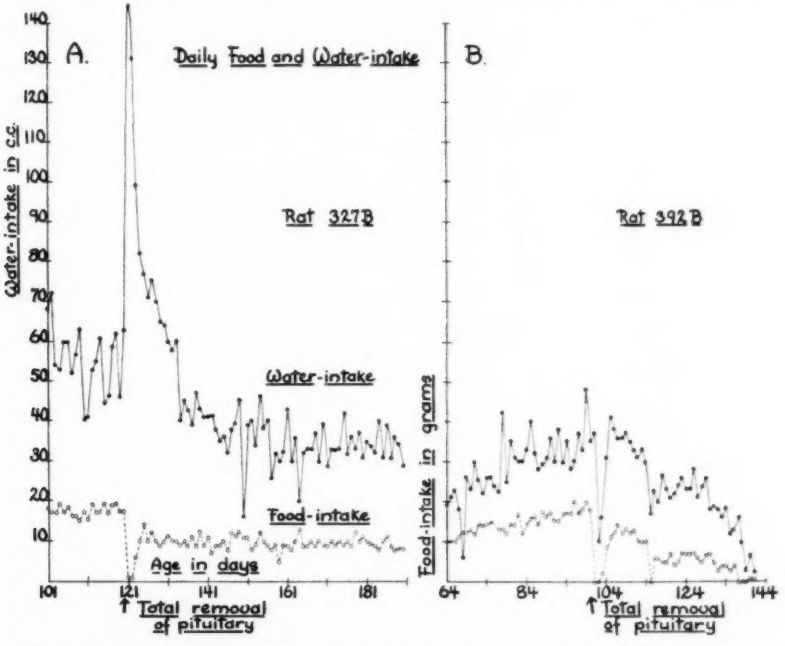

Fig. 1A. Graph showing temporary increase in water-intake and permanent decrease in food-intake produced in rat 327 B by total removal of the pituitary gland. Ordinates: water-intake in cubic centimeters; food-intake in grams. Abscissae: age in days.

1B. Graph showing oliguria and decreased food-intake produced in rat 392 B by total removal of the pituitary gland.

complete hypophysectomy. One rat, no. 596, the only one showing a permanent diabetes insipidus, was transferred to the partial list, despite the fact that no glandular tissue was found at autopsy, because the body weight curve showed no change. From our experience and that of Smith this would indicate very definitely that some anterior lobe tissue must have been left in the animal. It is possible that the remnant was hidden in the dural sac and was missed at autopsy.

Total removal of the hypophysis, then, did not produce permanent diabetes insipidus in a single instance.

Partial removal. The animals with partial removal of the hypophysis were of two different groups, those in which all of the posterior lobe was

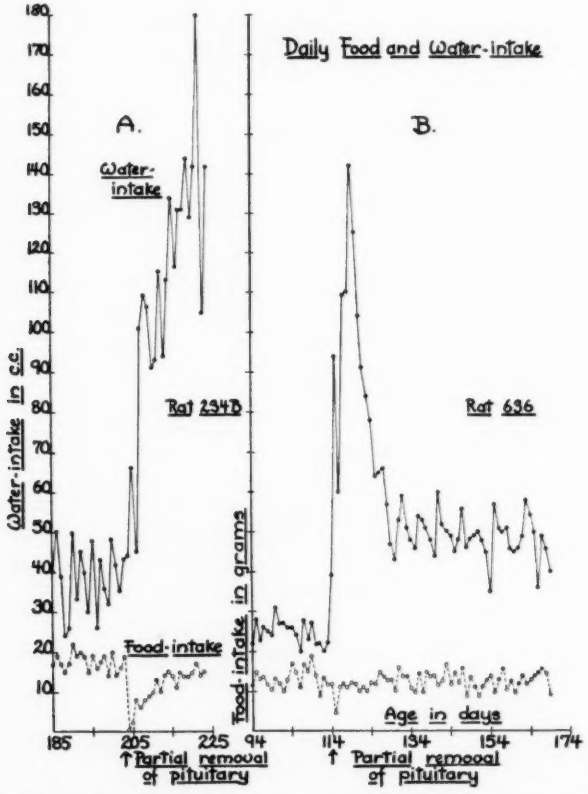

Fig. 2A. Graph showing permanent increase in water-intake produced in rat 234 B by partial removal of the pituitary gland.

2B. Graph showing permanent increase in water-intake produced in rat 636 by partial removal of the pituitary gland.

removed while part of the anterior lobe remained, and those in which parts of both anterior and posterior lobes remained.

Two typical curves of the twenty-six animals of the former group are presented in figure 2. In the first graph the operation is seen to have produced an immediate increase in water-intake from 40 cc. to 130 cc. per day, at which approximate level it was maintained until the animal was

killed 21 days later. It will be noted that the food-intake was approximately normal when the experiment was concluded.

The second graph shows a marked increase in water-intake and then a gradual drop. However, in contrast to the curves of the complete removal, it did not continue to drop to the original level, but stopped at 50 cc. per day, 25 cc. above the pre-operative average, at which level it remained until the animal was killed.

The twenty-six animals with part of the anterior lobe intact but no posterior lobe, showed a consistent, definite, and permanent increase in water-intake. In all but two or three animals, the increase was marked, with the greatest change recorded from 38.1 to 137 cc. There were no consistent changes in food-intake; some animals showed none at all, others an increase, and the rest a small decrease. The majority showed a negligible decrease compared to that found in the animals with complete hypophysectomy. In the latter group the food-intake averaged 14.1 grams before and 6.2 grams after the operation; in the partially hypophysectomized group, there was no significant change, the pre- and post-operative averages being 12.7 and 12.2 grams respectively.

Of the six animals in which parts of the posterior lobe as well as the anterior lobe were found, three showed a small decrease rather than an increase in water intake while the other three showed a rise which would have occurred during this time due to growth phenomena. It may be said that here the presence of the posterior lobe prevented increased thirst.

It is of considerable importance to note that in three of these animals part of the posterior lobe was found still connected to the stalk. It is very likely that the connections were intact in the other three animals also, but due to the autopsy methods used in these instances this fact could not be confirmed. Emphasis is put on this point because, as will be shown below, it is not the removal of the posterior lobe which is important but the severance of its connections with the stalk.

There is some evidence to indicate that the final level of water-intake after partial hypophysectomy seems to depend, in direct ratio, on the amount of the anterior lobe that remains. In addition, it makes some difference whether the stalk connections of the anterior lobe to the brain are still intact or not. Thus at autopsy it was found that the animal with the permanently very high water-intake, whose curve is shown in figure 2A, had small but intact connections between the anterior lobe and the brain stem, whereas the animal with the permanent but less intense polydipsia, whose curve is shown in figure 2B, had no stalk connection and only a very small fragment of anterior lobe tissue.

Thus we see that none of the animals with a permanent diabetes insipidus had posterior lobe tissue but they all had anterior lobe tissue; whereas the animals with the temporary diabetes insipidus were without both anterior and posterior lobe.

These results would seem to warrant the conclusion that the necessary factor for the production of diabetes insipidus is the removal of the posterior lobe, and that the permanence of the condition depends on the presence of anterior lobe tissue. Such a conclusion is contrary to the belief that the permanence of diabetes insipidus is somehow determined by the site of the lesion in the brain stem.

The observation that a permanent diabetes insipidus did not occur in the absence of anterior lobe tissue confirms the findings of von Hann made in 1918 in a study of autopsy records on patients with diabetes insipidus. He found that twenty patients suffering from this illness had lesions of the posterior lobe with parts of the anterior lobe still intact while nine patients with much the same lesion but with the anterior lobe gone showed no diabetes insipidus.

Discussion. On the basis of these experiments and others reported previously, a few general conclusions may be drawn regarding the factors involved in the production of diabetes insipidus. In the first place, both the anterior and posterior lobes of the pituitary gland play an important part in the production of this phenomenon, the anterior lobe apparently determining the permanence of the diabetes insipidus, and also possibly the intensity. Such a function can be explained in one of two ways. In the first place, on the part of the anterior lobe a diuretic substance produced in the anterior lobe and ordinarily counterbalanced by an antidiuretic substance in the posterior lobe would explain the results of the above experiments. Dr. E. M. K. Geiling (unpublished) has recently obtained some evidence indicating the presence of such a diuretic substance in the anterior lobe. Or, and this explanation seems more plausible, the anterior lobe may maintain the normal metabolism, growth, activity, etc., and without it the decrease in metabolism and general activity may be so great that all of the needs of the organism are reduced practically to a minimum. An animal under such circumstances would become almost totally inactive; it would lose weight due to loss of appetite; and in the same way its water-intake would be reduced to a physiological minimum. That water-intake does follow very closely the changes in general bodily metabolism is shown by the fact that the onset and rate of decrease in water-intake following total hypophysectomy are the same for water-intake as for food-intake and body-weight.

There seems to be little doubt that the importance of removal of the posterior lobe lies in the elimination of the anti-diuretic substance produced there. The chief evidence for this statement lies in the fact that, in confirmation of van de Velden's original observation (1913), injection of posterior lobe extract, pitressin or pituitrin, reduces the water-intake of even the best polydipsic animals almost at once to normal and maintains it there as long as the injections are continued.

Our knowledge of the site of action of pituitrin in polyuric animals is still very limited. It may act directly on the kidneys; it may act on the body tissues in general or the mucous membranes of the throat in particular; or it may act on the choroid plexus. Recent work of Burgess, Harvey and Marshall (1933) has shown that pituitrin acts on the kidney by contracting the loops of Henle. However, this may not be the only effect in a diabetic animal.

It is important to note that a large increase in water-intake can be obtained simply by section of the stalk between the posterior lobe and the brain, or by total removal of the posterior lobe. It was found in experiments in which lesions were made with a small scalpel that the greatest effect could be produced by a stab wound made near the anterior border of the pituitary gland so as to sever the stalk but leave the posterior lobe intact (Richter, 1930). The significance and mode of operation of such a lesion are not clear. Section of the stalk must somehow either prevent the posterior lobe secretions from reaching their site of action, wherever that is in the body, or block the stimulation to the hypophysis itself, thus stopping the production of pituitrin. In the former instance stalk section would block the passage of the secretion either into the blood stream or into the third ventricle; in the latter instance the passage of nervous impulses from the supra-optic nuclei in the brain-stem to the posterior lobe would be interrupted.

Experimental results reported by a number of other workers are not in agreement with our belief in the importance of injury to the posterior lobe during stalk section, in the production of diabetes insipidus. Bailey and Bremer (1921) in particular, report that they were able to produce an intense diabetes insipidus in dogs by a lesion in the brain-stem, completely sparing the hypophysis. We have found in a detailed study of this region of the brain in forty dogs, that it is very difficult to lift the brain from the base of the skull without rupturing the posterior lobe connections in the stalk and also the leptomeninges in the infundibular region. This is in agreement with the observations of Hanchett (1922) that polyuria can be produced very consistently by traction on the stalk alone. On the basis of our observation there seems to be little doubt that the posterior lobe connection to the brain was severed in all dogs described by Bailey and Bremer as showing a diabetes insipidus.

It would seem then that the essential and only factor necessary for the production of diabetes insipidus is a lesion severing the connection between the posterior lobe and the nuclei of the brain stem, provided, of course, that some anterior lobe tissue remains. There are, however, several facts which can not be fitted easily into such a simple formulation. Most difficult to explain is the observation that total hypophysectomy does not consistently produce diabetes insipidus (Cushing, 1932; Camus and

Roussy, 1913; Dandy and Reichert, 1925). The fact that it produces diabetes insipidus in some animals and not in others, would seem to indicate that some additional factor must be involved; that during the removal of the gland, other structures were injured.

An extensive search has been made to locate this additional factor. In view of the theories held by many workers regarding the significance of brain injuries in furthering the production of diabetes insipidus the obvious suggestion would be that the brain stem was injured in animals showing diabetes insipidus, and spared in those which did not show it. Observations from many different angles have led us to the conclusion that the brain injury did not contribute to the production of the diabetes insipidus. In the first place, we have found in a histological study of brain sections that some of our animals with the most intense and prolonged polydipsia had no detectable lesion in the brain stem; furthermore, since the pituitary can be removed from the rat by suction through the sphenoid bone so that all contact with the brain stem is eliminated, there can be no question of a brain stem injury in the totally hypophysectomized rat showing diabetes insipidus. Finally, it was found that lesions made in all parts of the brain never produced a diabetes insipidus unless posterior lobe stalk connections were severed.

A study of this region disclosed another factor which we feel may finally prove to be significant in the production of diabetes insipidus. It was found that in the rat, and in most animals including man, the third ventricle comes to the surface of the brain in several places near the infundibulum, in one place in particular just posterior to the infundibulum, between the mammillary bodies (Richter and Benjamin, 1934). In this region an injury to the meninges is apt to rupture the floor of the ventricle and permit the free escape of cerebro-spinal fluid. The possible significance of such an injury is suggested by the fact that with only two exceptions in over a hundred animals with diabetes insipidus, ink injected into the third ventricle diffused freely from a rent at some point in the leptomeninges. It will be noted that one of these delicate areas in the rat and also in the dog is in a position clearly subject to stress during hypophysectomy and that the other (area no. 1) is located exactly at a point where, according to Bailey and Bremer, lesions so minute as to be invisible to the eye, produced diabetes insipidus.

It is not clear at the present time how the rapid escape of cerebro-spinal fluid could contribute to the production of diabetes insipidus. However, the suggestion that the cerebro-spinal fluid may contribute to this phenomenon is not as extraordinary as it may appear when it is recalled that there are many instances on record in which diabetes insipidus has been relieved and even cured by lumbar puncture (Cammidge, 1920; Graham, 1917; Herrick, 1912; Tucker, 1922) and by air-injection (Schube, 1933).

Further experiments now in progress on this phase of the problem will be reported later.

SUMMARY

1. The pituitary gland was totally removed in thirty-four rats; partially, in thirty-two.

2. Total removal of the gland produced a temporary diabetes insipidus in twenty-eight animals, a permanent diabetes in none.

3. A permanent diabetes insipidus was found in twenty-six animals in which the posterior lobe was entirely removed but in which part of the anterior lobe remained.

4. Six animals in which parts of the posterior lobe remained (probably still in connection with the stalk) showed no diabetes insipidus. The degree of the permanent diabetes insipidus seems to depend on the amount of the anterior lobe remaining, and whether or not this lobe is still in connection with the pituitary stalk.

5. The present research regarding the importance of the anterior lobe in diabetes insipidus confirms the conclusion arrived at by von Hann in 1918 in a study of clinical material in man.

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PERIPHERAL CIRCULATION DURING EXPERIMENTAL FEVER

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Alteration of the flow of blood in the peripheral tissues constitutes a factor of foremost importance in the regulation of body temperature under normal and pathological conditions. In present knowledge there appear to be at least three physiological mechanisms which play important rôles in the development of fever. The first is peripheral constriction, by means of which heat loss may be decreased (Hewlett, 1911; Stewart, 1911; Fremont-Smith, Morrison and Makepeace, 1929); the second is the adrenal mechanism (adrenal medulla), which can augment heat production through the liberation of adrenine (Cannon, Querido, Britton and Bright, 1927); and the third is the shivering mechanism, which when thrown into activity is capable of producing heat at a very rapid rate. The investigation here reported was undertaken with the hope of securing additional information concerning the relative importance of peripheral vasoconstriction in febrile reactions.

METHOD. For several reasons the rabbit was selected as the experimental animal for these observations. The external ears serve as satisfactory extremities in which to study peripheral circulation because a fairly accurate estimation of the degree of vasoconstriction or vasodilatation can be obtained by merely observing the color of these parts. A more accurate procedure is that of determining the temperature of the ears by a thermoelectric method. The temperature of the ears is influenced only indirectly by the temperature of tissues situated more deeply in the body. For this reason the temperature of these appendages may be considered as a good index of peripheral vasomotor changes.

The possibility of destroying all sympathetic innervation of one ear without interfering with the innervation of the other was another reason for using the ears in these investigations. By sympathectomizing only one ear a preparation is obtained which, at the same time, provides both a control and an experimental test.

By means of thermocouples (copper-constantan) and a sensitive string galvanometer, records of temperature were obtained from both ears and the rectum at close intervals. One thermocouple was fastened to each ear by means of liquid adhesive. Another thermocouple was inserted into the

rectum and held in place by adhesive tape wrapped around the tail of the animal. By means of two multiple-connection switches it was possible to make a series of readings, one from each thermocouple, within a very short time (50–70 seconds).

In the rabbit the sympathetic innervation of the ear includes fibers from the superior cervical ganglion, and also from the stellate of the same side (Fletcher, 1898; Feldberg, 1926), reaching the ear by way of the auricular nerves. In some experiments the ear was sympathectomized by removal of the superior cervical ganglion and section of both auricular nerves on one side. In other experiments the sympathetic innervation to one ear was destroyed by removal of the stellate ganglion on that side. The superior cervical ganglion was removed in the former operation through a midline incision in the region of the larynx, and the auricular nerves were severed through a midline incision from the external occipital protuberance to the spinous process of the first thoracic vertebra.

The numerous difficulties associated with intrathoracic operations in the rabbit made desirable the removal of the stellate ganglion without entering the pleural cavity. This was accomplished most satisfactorily by a dorso-lateral approach. The animal was anesthetized with ether and fastened, ventral surface downward, on an animal holder, a narrow, trough-like board mounted on a wooden stand. The legs of the rabbit were drawn downward and fastened in the extended position. A skin incision was made in the midline from about the fourth cervical vertebra to the fourth or fifth thoracic. The muscles attaching the scapula to the spinous processes were sectioned on one side and the scapula then drawn downward. The muscles overlying the first intercostal space, about 1 to 2 cm. lateral to the vertebral column, were separated and retracted, thus exposing the first and second ribs near their vertebral junctions. The muscles between the first and second ribs were torn by blunt dissection, from the vertebral junction laterally for about 2 to 3 cm., with care not to break into the pleural cavity. The first and second ribs were then spread apart slightly. Although there are numerous thin-walled veins in this region, it was possible to approach the stellate without much hemorrhage, provided the dissection was done with caution. Then the stellate ganglion could usually be picked up with comparative ease. After it had been removed the muscle layers, connective tissue and skin were approximated and held in place by silk ligatures. The success of the operation could be determined, after the animal had recovered from the anesthesia, by the extent to which the blood vessels of the homolateral ear were dilated.

In some animals the adrenal glands were inactivated in order to eliminate the factor of adrenine secretion during the febrile reactions. This was accomplished by removal of the right gland and denervation of the left by section of the splanchnic nerves and removal of the sympathetic chain from the diaphragm to the fourth or fifth lumbar vertebra.

All experiments were performed on unanesthetized animals, after they had recovered completely from the effects of the surgical operations. Fever was produced by the intravenous (marginal ear vein) injection of typhoid-paratyphoid vaccine.[1] It was found that even in the normal state some rabbits were completely refractory to the vaccine; others, however, gave excellent febrile responses. In the earlier experiments of this series the animals were comfortably fastened to an animal board and kept in a large insulated box throughout a single experiment. Even though the temperature of the box was maintained fairly high (26 to 30°)[2] during the experiment, the immobile animals lost body heat rapidly. For this reason a number of observations were carried out on unleashed animals. In these experiments the rabbits were fastened to the animal board only during the time required to apply the thermocouples. Thereupon they were set in a small, oblong box, where they remained fairly quiet. In order to assure ample ventilation the box was kept open at the top except for several narrow leather straps. The vaccine was not injected until at least 30 minutes after the animal had been placed in the box.

As stated previously, some rabbits failed to develop fever after the injection of vaccine. In such instances the experiment was discontinued and the animal discarded. No definite relationship was detected between the amount of vaccine injected and the severity of the febrile reaction. The usual dose employed was 0.25 to 0.50 cc. per kgm. body weight. After the first injection there appeared to develop in some animals a slight refractoriness to subsequent injections; in a few instances a second injection evoked no febrile response.

At the conclusion of the experiments the animals were sacrificed. A post-mortem examination was made in order to verify the success of the surgical operations performed.

Results. After section of all sympathetic nerves of the rabbit's ear a marked dilatation of the blood vessels occurred throughout the entire structure. Although the degree of vasodilatation became somewhat less within one or two weeks after operation, the effect was still detectable at the end of one or two months.

When an animal in which one ear had been sympathectomized was given typhoid-paratyphoid vaccine intravenously, there occurred usually a prompt vasoconstriction in the normal ear, as indicated by a pronounced decrease in its temperature. The extent of the decrease depended, of course, upon the condition of the ear vessels just before the injection, and upon the environmental temperature. During the constriction of

[1] A vaccine supplied by the Massachusetts Toxin-Antitoxin Laboratories, containing a billion killed typhoid organisms and a billion and a half killed paratyphoid organisms per cubic centimeter.

[2] All temperatures are given in degrees centigrade.

the vessels of the normal ear there occurred occasionally a pronounced vascular constriction also in the sympathectomized ear. In 2 of 9 experiments on rabbits in which the adrenals were intact, the temperature of the sympathectomized ear followed closely that of the normal ear after the injection of vaccine. In 3 instances there was a distinct decrease in the temperature of the sympathectomized ear, but the decrease came on relatively late in the febrile response. An experiment in which this occurred is represented by figure 1. In 4 experiments there was no appreciable decrease in the temperature of the sympathectomized ear, even though the normal ear exhibited a striking decrease. Figure 2 shows the results from a typical experiment in this group. After the injection of

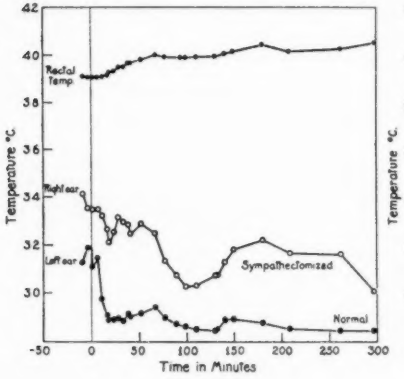

Fig. 1

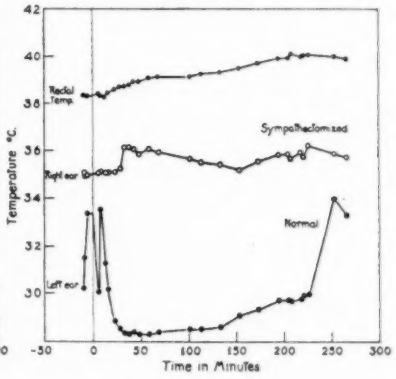

Fig. 2

Fig. 1. Changes of temperature of the normal and the sympathectomized ear after the injection of typhoid-paratyphoid vaccine (exp. 26). Adrenals intact. Vaccine injected at 0.

Fig. 2. Changes of temperature of the normal and the sympathectomized ear after the injection of typhoid-paratyphoid vaccine (exp. 29). Adrenals present. Vaccine injected at 0.

vaccine there occurred a typical febrile response; the animal shivered vigorously, there was a pronounced decrease in the temperature of the normal ear and an increase of the rectal temperature. Nevertheless, the sympathectomized ear showed no decrease of temperature; in fact, there was a slight increase.

In table 1 is given a summary of the results obtained from the experiments in which the adrenals were present. After the injection of vaccine there was always a marked decrease in the temperature of the normal ear. In experiments 12, 26 and 35 the decrease was somewhat less than in the other experiments, because even before the injection of vaccine the temperature of the normal ear was rather low. In only 4 of 9 experiments

was there a marked decrease in the temperature of the sympathectomized ear after the injection. A slight increase occurred in 2 instances. In experiment 44, in which the rectal temperature increased 2.21°, the temperature of the sympathectomized ear did not decrease, even though the normal ear decreased 6.63°.

The delayed vasoconstriction in the sympathectomized ear in experiment 26 (fig. 1) suggests that some humoral mechanism was thrown into activity at the height of the febrile reaction. Within a few minutes after the injection the sympathectomized vessels showed a slight vasoconstriction, but it did not become pronounced until almost two hours later. In the experiments in which the temperature curve for the sympathectomized ear followed closely that for the normal ear after the injection of vaccine,

TABLE 1

Variation of temperature in ears of rabbits after injection of typhoid-paratyphoid vaccine

(Adrenals intact; one ear sympathectomized and one normal)

EXPERIMENT NUMBER	ANIMAL NUMBER	INITIAL RECTAL TEMPERATURE	MAXIMAL INCREASE OF RECTAL TEMPERATURE	INITIAL TEMPERATURE OF NORMAL EAR	MAXIMAL DECREASE OF TEMPERATURE IN NORMAL EAR	INITIAL TEMPERATURE OF SYMPATHECTOMIZED EAR	MAXIMAL DECREASE OF TEMPERATURE IN SYMPATHECTOMIZED EAR
		°C.	°C.	°C.	°C.	°C.	°C.
12	T31	38.10	0.96	29.58	2.53	31.52	3.84
23	T40	38.35	1.75	34.30	8.08	33.78	7.57
26	T42	39.06	1.47	30.59	2.15	34.27	4.22
29	T40	38.33	1.79	33.35	5.05	34.98	No decrease
30	T45	38.42	1.65	36.57	5.92	35.64	0.37
35	T47	37.39	0.85	28.00	1.07	34.64	5.06
44	T35	39.03	2.21	35.97	6.63	36.46	No decrease
47	T35	38.27	1.53	36.60	7.96	36.34	0.18
48	T36	38.42	3.11	37.01	9.01	36.29	0.81

it was suspected that the adrenal medulla had been excited to activity at the beginning of the febrile reaction. The delayed vasoconstriction which occurred in the sympathectomized ear in experiment 26 (fig. 1) proved, however, a rather baffling riddle.

In an attempt to throw additional light on the phenomenon of delayed vasoconstriction, several experiments were performed on animals in which the adrenals had been inactivated. In figure 3 may be seen the results of one of them. Although the adrenals were inactivated, there was a pronounced vasoconstriction in the sympathectomized ear; but it was much delayed, occurring about one and a half hours after the vaccine was injected. Noteworthy is the fact that with the onset of vasoconstriction in the sympathectomized ear there was an increase in the vasoconstriction already present in the normal ear. Another point of interest in

connection with this experiment was the failure of the vessels of the sympathectomized ear to dilate normally when the fever began to break. This fact is clearly illustrated in figure 3. The failure of normal vasodilatation in this case, however, might be explained by the fact that in cutting the auricular nerves, not only were vasoconstrictor fibers (from the stellate ganglion) destroyed, but also the majority of the vasodilator fibers to the ear. Later experiments yielded results which tend to show that this explanation is only partially correct.

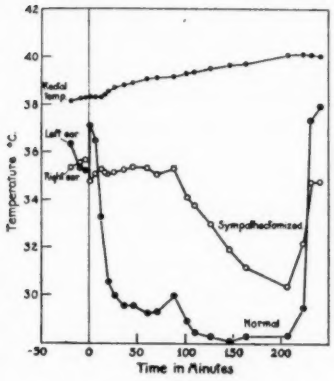

Fig. 3

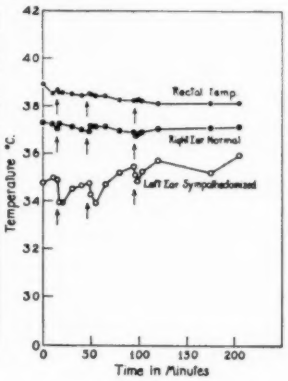

Fig. 4

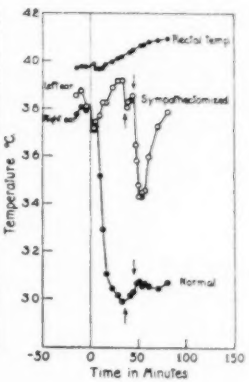

Fig. 5

Fig. 3. Changes of temperature that occur in the normal and may occur in the sympathectomized ear after the injection of typhoid-paratyphoid vaccine (exp. 16) in animals with adrenals inactivated. Vaccine injected at 0.

Fig. 4. The effect of struggle on the temperature of the normal and the sympathectomized ear (exp. 33). Adrenals present. Arrows indicate the times at which struggling occurred.

Fig. 5. Alteration of temperature in the normal and the sympathectomized ear after the injection of typhoid-paratyphoid vaccine and adrenalin chloride (exp. 49). Adrenals present. Vaccine injected at 0. Arrows indicate times at which adrenalin chloride was injected.

In table 2 is given a summary of the results of all experiments on animals with inactive adrenals. In 1 instance there was no constriction of the vessels in the sympathectomized ear; in 2 instances there was a pronounced constriction. In no experiment, however, was there an immediate constriction of the sympathectomized vessels after the injection of vaccine. On the basis of these results it may be concluded that the delayed constriction observed in 3 of the experiments in which the adrenals were intact was not the result of liberation of adrenine into the blood stream, but perhaps some other humoral substance liberated late in the febrile reaction. Adrenal stimulation was presumably responsible for the vasoconstriction of the sympathectomized vessels which in 2 instances occurred promptly after the injection of vaccine.

Often during these experiments it was observed that when the animal struggled there was a more pronounced constriction of the vessels in the sympathectomized than in the normal ear. An experiment was carried out in order to study this phenomenon more carefully. The results (fig. 4) confirm those of other investigators, namely, that destruction of the sympathetic supply of a structure renders that structure more responsive to adrenine (Elliott, 1905). This fact is further borne out by the results obtained in experiment 49 (fig. 5), in which, after injection of typhoid-paratyphoid vaccine had failed to cause constriction of the vessels in the sympathectomized ear, adrenalin chloride was injected intravenously. Although the first injection (0.25 cc. of adrenalin chloride, 1:100,000) caused only a slight constriction of the sympathectomized vessels (no constriction in the normal), a larger dose (1 cc.) caused a very pronounced

TABLE 2

Variation of temperature in ears of rabbits after injection of typhoid-paratyphoid vaccine

(Adrenals inactive; one ear sympathectomized and one normal)

EXPERIMENT NUMBER	ANIMAL NUMBER	INITIAL RECTAL TEMPERATURE	MAXIMAL INCREASE OF RECTAL TEMPERATURE	INITIAL TEMPERATURE OF NORMAL EAR	MAXIMAL DECREASE OF TEMPERATURE IN NORMAL EAR	INITIAL TEMPERATURE OF SYMPATHECTOMIZED EAR	MAXIMAL DECREASE OF TEMPERATURE IN SYMPATHECTOMIZED EAR
		°C.	°C.	°C.	°C.	°C.	°C.
13	T32	38.38	0.39	36.25	9.34	32.77	0.72
14	T34	38.05	1.82	31.89	4.60	38.40	4.62
16	T34	38.27	1.84	35.20	7.85	35.66	5.29
45	T41	38.77	2.52	36.80	8.32	37.33	0.80
46	T41	38.86	1.40	37.34	7.73	33.16	No decrease

constriction. Even with the larger dose, however, there was no appreciable change in the normal vessels.

Evidence for the existence of vasodilator fibers in sympathetic nerves has been presented by several investigators within recent years (Hoskins, Gunning and Berry, 1916; Lewis and Pickering, 1931; Cannon and Rosenblueth, 1933). In many of the experiments reported in this paper it was observed that during periods when there was extreme vasodilatation in the normal ear, the vessels of the sympathectomized ear were only partially dilated. This difference was mentioned in connection with experiment 16 (fig. 3). In that instance, however, the ear had been sympathectomized by removal of the superior cervical ganglion and by section of the auricular nerves. To attribute the effect solely to removal of sympathetic innervation was therefore impossible. That the same phenomenon was observed in animals in which the sympathetic supply of the ear vessels had been

abolished by removal of the stellate ganglion, however, suggests that the failure of normal vasodilatation was due principally to destruction of the sympathetic innervation.

It was stated above that during some of these observations the experimental animal was fastened to an animal board; in others the animal was not leashed. The only difference apparent between the results obtained from the two groups was that the increase of rectal temperature, after the injection of vaccine, usually was greater in the unleashed animals. These animals, even though inactive during the experimental period, did not lose body heat as rapidly as did the immobilized animals which were stretched out.

The experimental results obtained from animals in which the ear vessels had been sympathectomized by removal of the stellate ganglion were not different from those obtained from animals in which the sympathetic innervation had been abolished by removal of the superior cervical ganglion and section of the auricular nerves.

Discussion. The results presented in this paper confirm the view that constriction of peripheral blood vessels must be considered as a normal occurrence in at least one type of febrile reaction, namely, that resulting from the injection of typhoid-paratyphoid vaccine. During the febrile reaction caused by the injection of this vaccine in rabbits, there was always a marked constriction of the blood vessels in the normally innervated ear. An interesting observation in this connection was that even though frequent and pronounced fluctuations in the temperature of the normal ear had been occurring prior to the injection, after the injection there developed immediately a maintained vascular constriction (with the resulting decrease of temperature) which often lasted for 2 hours or more.

There appear to be several mechanisms by which this peripheral vasoconstriction may be brought about. The most important of these, under normal conditions, is the sympathetic innervation of the vessels. In several of the present experiments after destruction of the sympathetic supply of the vessels of one ear there was no constriction of those vessels during the febrile reaction. Another mechanism which must be considered is the adrenal medulla. The results obtained from these experiments suggest, however, that with a normal sympathetic innervation, the activity of the adrenal medulla plays no significant rôle in bringing about peripheral vasoconstriction.

Attention has been called to figure 5, which represents an experiment during which adrenalin chloride was injected intravenously. The normally innervated vessels failed to constrict after the injection of 1.0 cc. of adrenalin (1:100,000), but the sympathectomized vessels showed a pronounced constriction. With the injection of 0.25 cc. there was a slight constriction in the sympathectomized ear and none in the normal ear. This confirms

the recent work of Freeman, Smithwick and White (1934), who showed that in man destruction of the sympathetic innervation of the vessels of the skin rendered those vessels more sensitive to adrenine. They also found sensitization in the sympathectomized vessels of the rabbit's ear.

On the basis of the results mentioned above we are led to conclude that in experiments 12 and 23 (table 1) stimulation of the adrenals during the febrile reaction led to the liberation of adrenine, for in each instance the temperature curve of the sympathectomized ear followed closely that of the normal side. In these experiments it might be suspected that the vessels of the ear were not completely sympathectomized. In opposition to such an opinion are the following facts: 1, after the operation for removal of the sympathetic innervation there was extreme dilatation of the vessels in the homolateral ear; 2, during the preliminary period before the injection of vaccine the temperature of the sympathectomized ear was much more constant than that of the normal—in other words, the normal ear showed its characteristic fluctuations of temperature, whereas the sympathectomized ear remained fairly constant; 3, post-mortem examination showed no indication of intact sympathetic fibers to the ear on the operated side.

Granted that in the two experiments mentioned above, 12 and 23, the liberation of adrenine was responsible for the constriction of the vessels in the sympathectomized ear, how is the failure to obtain such constriction in experiments 29, 30, 44 and 47 (table 1) to be explained? In each of these experiments a marked decrease of temperature occurred in the normal ear, but no appreciable decrease in the sympathectomized ear. The failure to obtain constriction in the sympathectomized vessels may have been due to a relatively low sensitivity to adrenine in these structures. This view is supported by the results of experiment 49 (fig. 5), which have been discussed above. Another possibility is that the sympathectomized vessels failed to constrict because there was no adrenine, or too little, liberated even though some sympathetic stimulation occurred, as evidenced by the constriction of the vessels in the normal ear. Still another possibility is that the height of general blood pressure was sufficient to maintain a dilated state in the denervated vessels in spite of the tendency of adrenine to cause constriction.

It is known that accessory tissue similar to that of the adrenal medulla is often present in the rabbit (Vincent, 1925), but little is known regarding its innervation. It may be that the delayed constriction observed in the sympathectomized vessels, even after inactivation of the adrenals, was caused by the liberation of adrenine from accessory medullary tissue. If this was not the case we must conclude that the constriction was caused by some humoral substance other than adrenine. Although it is purely hypothetical to postulate an involvement of the pituitary in this phenome-

non, one is inclined to wonder if the delayed constriction would still occur after removal of this gland.

SUMMARY

1. The febrile reaction in rabbits which results from the injection of typhoid-paratyphoid vaccine invariably causes a marked constriction of the blood vessels in the normally innervated ear, as indicated by temperature changes. This constriction comes on within a short time (5 to 10 minutes) after the injection and persists until the "peak" of the temperature rise has developed. In the sympathectomized ear constriction occurs in only about 50 per cent of the cases (see table 1, and figs. 1 and 2).

2. In some of the instances in which there is vasoconstriction in the sympathectomized ear the constriction takes place almost immediately after the injection of vaccine; in others the constriction is much delayed (1 to 3 hours after the injection).

3. The delayed vasoconstriction in the sympathectomized vessels may not be abolished by inactivation of the adrenals (see table 2, and fig. 3); the constriction which comes on immediately after the injection of vaccine, however, is abolished by inactivation of the adrenals.

4. Evidence is presented which indicates that destruction of the sympathetic innervation to the vessels of the ear renders these vessels more sensitive to adrenine than are the normally innervated vessels (see fig. 5).

5. The blood vessels of the sympathectomized ear do not dilate so completely as do those of the normal ear when there is general peripheral vasodilatation (i.e., when body temperature is decreasing after a febrile response, see fig. 3).

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THE QUANTITATIVE AND QUALITATIVE OVARIAN RESPONSE TO DISTRIBUTED DOSAGE WITH GONADOTROPIC EXTRACTS

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It was observed that both the nature and the degree of the ovarian response to hypophyseal gonadotropic extracts are markedly affected when various salts or protein precipitants are incompletely removed. An investigation of the effect of distributed dosage indicated that these substances markedly retard the rate of absorption of the hypophyseal principle in the tissues. By employing distributed dosage with the addition of zinc sulfate to the extracts to regard absorption in the tissues, results have been obtained which suggest that the wide variation in ovarian response observed with different hypophyseal preparations may be attributed solely to differences in physical properties which affect their rate of absorption.

EXPERIMENTAL. *Preparations and methods.* The sheep and beef pituitary preparations were made according to the method of Van Dyke and Wallen-Lawrence (1933) and the urine of pregnancy preparation according to the procedure of Marshall (1932). The same preparations were used throughout.

Biological assay was carried out on 21 to 23 day old female albino rats. The animals, except when otherwise stated, were dosed daily for four days and sacrificed on the 6th day. Litter mate controls were used in comparative experiments. In studying the effect of distributed dosage the single daily dose (0.5 cc.) of the extract was diluted and given in 0.5 cc. volume in repeated injections throughout the day. When $ZnSO_4$ was employed, the total dosage per animal given daily was approximately 3 mgm. The calculated amount of a 10 per cent $ZnSO_4$ solution was added to the solution containing the hypophyseal powder and the reaction immediately adjusted to pH 5.7 ± 0.2. In all comparative experiments the solutions were prepared and kept under identical conditions.

The effect of distributed dosage upon the quantitative ovarian response to hypophyseal gonadotropic extracts. It was observed that the presence of small amounts of zinc salts in hypophyseal extracts produced a marked augmentation in the ovarian weight increase. Single daily injections of

the extract in the presence of zinc sulfate produced an even greater augmentation than could be obtained by administering the same amount of extract alone, divided into six doses daily. Thus in a series of six 21–23 day old rats, given single daily injections of 3 mgm. of the sheep hypophyseal preparation, the average ovarian weight on the 6th day was 25 ±2 mgm. A series of six litter-mate controls receiving the same amount divided into six doses at three-hour intervals daily, showed an average ovarian weight of 55 ±4 mgm. However, single daily injections of one and two milligrams of the same extract, to which had been added 3 mgm. of $ZnSO_4$, given two groups of four animals in the same age group produced ovaries on the 6th day averaging 46 mgm. and 106 mgm. respectively. When divided dosage was employed with hypophyseal extracts containing zinc sulfate, an even greater augmentation in the ovarian weight resulted. Thus, 1 mgm. daily of the preparation containing zinc sulfate when given in two injections produced on a group of four animals ovaries averaging 170 mgm., while 0.25 mgm. daily, divided into four injections, on a group of six animals gave ovaries on the 6th day averaging 52 ±4 mgm.

In isolated experiments a comparable ovarian weight augmentation was obtained by the addition of tungstic acid or of aluminum salts to hypophyseal extracts. That the action of these compounds as well as that of zinc sulfate consists entirely in delaying absorption was shown by separate injection of the retarding agent. In this event no augmentation resulted. It was also observed that upon removal of the retarding agent the preparation showed only its approximate original potency.

The ovarian response to distributed minimal dosage with hypophyseal extracts. Crude hypophyseal extracts whose minimal response was the production of a few corpora, upon the addition of $ZnSO_4$ were found with minimal dosage to exert a strong estrogenic action upon the uterus, often without evidence of morphological changes having occurred in the ovaries. In a series of twelve 21–23 day old rats given in single daily injections sufficient sheep hypophyseal extract (0.12 mgm. daily) in combination with $ZnSO_4$ to produce an estrous uterus and rupture of the vaginal membrane in 100 hours, the ovarian weight on the 6th day averaged 15 ±2 mgm. Upon section of the ovaries, six of the group were found to contain only small and a few medium sized follicles and could not be definitely distinguished from the ovaries of undosed controls in the same age group. The ovaries of the remainder showed evidence of some follicular development but without the occurrence of luteinization. When an equivalent amount of the same preparation without the addition of zinc sulfate was given to four animals in the same age group, the uterus remained thread-like and the ovaries infantile.

In order to determine whether more prolonged dosage would result in definite changes in the ovaries, minimal dosage with the sheep hypophyseal

preparation containing $ZnSO_4$ was continued for a period of ten days on six animals. Rupture of the vaginal closing membrane with a pro-estrous smear which changed to cornified occurred on all of this series between 100 and 120 hours. The ovaries on the 11th day averaged 15.4 ±2 mgm. Two of the group showed ovaries which upon section could not be distinguished from controls. Three showed ovaries containing follicles in all stages of development, while one contained one small corpus luteum.

Since the minimal response to beef anterior lobe preparations has invariably been the production of a few corpora lutea, the effect of the addition of zinc salts upon it was next investigated. A crude beef preparation was used, which with single daily injections of 5 mgm. and 10 mgm. for four days poduced ovaries averaging 18 mgm. and 33 mgm. respectively. This dosage on two series of four animals produced corpora in the ovaries of all the animals. Slight uterine hypertrophy was evident on the larger dosage but the vagina was closed on all of the animals at autopsy. In the presence of zinc sulfate, 1 mgm. of the beef preparation daily to a group of four animals produced an estrous uterus with rupture of the vaginal membrane in 100 to 120 hours. The weight of the ovaries averaged 23 mgm. and upon section all showed follicles in all stages of development but no corpora lutea.

Maximum uterine stimulation was observed in a number of isolated experiments with sheep preparations contaminated with zinc salts before the significance of the zinc was understood. In frequent instances it was not possible to distinguish microscopically between the ovaries of the dosed and control animals. In going over data collected on several hundred animals given single daily injections of similar pituitary preparations, it was found that in the absence of retarding agents vaginal canalization rarely occurred by the 6th day unless the ovarian weight exceeded 35 mgm. When rupture of the vaginal membrane occurred corpora lutea were present in every instance examined.

The production of a follicular ovarian response by distributed dosage with hypophyseal extracts containing $ZnSO_4$. Since at low dosage levels single daily injections of the hypophyseal preparation in combination with $ZnSO_4$ almost invariably produced little or no evidence of luteinization, the effect of further increasing and distributing the dosage was investigated. In two groups of six animals given four injections daily of the extract containing $ZnSO_4$, the ovarian weights averaged 52 ±4 mgm. and 82 ±8 mgm. The ovaries of three in the first group and two in the second group showed a pure follicular response. In another series of five given three injections at five-hour intervals daily for three days and sacrificed at 100 hours, three in which the ovaries weighed 45, 65 and 69 mgm. failed to show evidence of luteinization. In another series of six animals dosed twice daily for ten days the ovaries of two, weighing 31 and 35 mgm.,

showed only follicular development. Corpora were present in the ovaries of the remaining animals in each group. At higher dosage corpora were always found when the ovarian weight exceeded 100 mgm.

In one series of six animals and in three groups of four animals given increasing amounts of the same preparation without the addition of $ZnSO_4$, the ovaries averaging 25, 38, 47 and 70 mgm. respectively, without exception showed luteal development.

That the action of the zinc salts consisted entirely in delaying absorption was shown by their removal from a preparation giving a follicular response. Thus one preparation containing $ZnCl_2$ when given in 0.2 cc. and 0.4 cc. doses produced follicular ovaries weighing 12 mgm. and 25 mgm., and accompanied by marked uterine development with rupture of the vaginal closing membrane. After removal of zinc by precipitation of the active principle with 80 per cent alcohol, a daily dosage equivalent to 0.7 cc. gave luteinized ovaries weighing 20 mgm. The vaginal closing membrane remained intact and only slight uterine hypertrophy was evident. It was further observed that after removal of zinc salts vaginal canalization did not again occur until the ovarian weight exceeded 35 mgm. Corpora lutea were then always macroscopically visible.

The effect of the addition of zinc sulfate to urine of pregnancy preparations. Evans (1929) has reported that the ovarian response to urine of pregnancy preparations is identical regardless of whether it is injected in single or in distributed daily dosage. In agreement with this we have found the addition of zinc sulfate to salt free urine of pregnancy preparations to be without effect upon either the degree or the nature of the ovarian response. The results with several different preparations upon a considerable number of animals were uniformly negative, and the data have therefore not been tabulated.

The effect of the addition of zinc salts to the hypophyseal synergistic fraction. It was found that amounts of hypophyseal extract which when given concurrently with urine of pregnancy preparations produced little or no augmentation (18 animals), upon the addition of zinc salts produced ovaries significantly greater (100 to 200 per cent) than the calculated expectancy (17 animals). In these experiments the crude sheep hypophyseal preparation used in previous experiments was used. With the exception of one experiment dosage was administered in single daily injections. In this experiment dosage with the hypophyseal fraction containing $ZnSO_4$ was divided into two injections daily.

Discussion. Fevold et al. (1933) and Wallen-Lawrence (1934) have presented evidence for the separation of a follicle stimulating principle from the hypophysis. Considerable of the experimental data of Fevold et al. was obtained by dosing their fraction twice daily as a colloidal tannate. This procedure they state accentuates the ovarian response without affect-

ing the qualitative results. This is contrary to our experience with extracts containing zinc salts.

As evidence of the separate existence of a luteinizing and a follicle stimulating principle Wallen-Lawrence (1934) reports that unfractionated hypophyseal extracts exposed to the action of formaldehyde produce at moderate dosage levels marked uterine and follicular stimulation without luteinization of the ovaries. Since a similar response may be obtained in the presence of zinc salts and since formaldehyde may alter the solubility of the proteins in the extracts its action can hardly be accepted as conclusive evidence of the destruction of a luteinizing principle.[1]

The production of follicular ovaries with distributed dosage of extracts containing zinc salts is admittedly not sufficiently consistent at higher dosage levels to discredit the dual hormone theory. However for our results to conform to such a theory it would be necessary to assume that zinc salts selectively retard the absorption of the luteinizing principle. In numerous experiments employing $Zn(OH)_2$ as an adsorbing agent no evidence has been obtained by us to indicate a selective adsorption of the luteinizing principle. Until additional evidence is acquired therefore our results appear more logically explained on the basis of the existence of a single sex stimulating principle.

SUMMARY

The potency of hypophyseal gonadotropic extracts may be increased as much as fifty-fold by sufficiently distributed dosage and the addition of zinc salts. Under these conditions the minimal response in immature rats is the production of an estrous uterus without the occurrence of morphological changes in the ovaries. A follicular ovarian response is uniformly obtained at low dosage levels. At higher dosage levels a follicular ovarian response is obtained in 25 per cent to 50 per cent of the animals when the ovarian weight does not exceed 100 mgm.

The observed augmentation with urine of pregnancy preparations is increased when zinc salts are added to the hypophyseal synergistic component.

That the action of zinc salts consists in retarding absorption of the active principle is shown by separate injection and by removal of the zinc.

The author is indebted to Dr. Richard D. Evans for examination of the microscopic sections and to Dr. Fritz Bischoff for advice in the prosecution of the work. We are also indebted to the International Cancer Research Foundation (Wm. H. Donner, Pres.) for a grant to

[1] Since this paper was submitted for publication a preliminary investigation has revealed that the ovarian response is not significantly accentuated by distributing the dosage with formalin-treated hypophyseal extracts.

study the effect of the pituitary upon neoplastic extension, of which this work is an outgrowth.

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THE EFFECT OF PERIODIC CHANGES IN AMPLITUDE OF RESPIRATION UPON BLOOD PRESSURE

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A study of graphic records of blood pressure shows that the smallest and most numerous of the changes observed are caused by the pulse wave transmitted to the arteries by ventricular systole. The waves of intermediate size normally observed correspond to the inspiratory and expiratory phases of respiration. Longer waves, regular or irregular in character, are frequently observed and are usually called vasomotor waves of blood pressure. The interpretation of the cause of some of these latter waves has not seemed sufficiently clear. Therefore, it was deemed advisable to study the effects of periodic changes in respiration upon the blood pressure, varying the amplitude of respiration within normal limits. During some preliminary work it was noticed that it is often possible to observe changes in blood pressure in animals, changes that seem to coincide with normal changes in the amplitude of respiration.

In this paper evidence is given to show that it is possible to produce changes in blood pressure which can be due to periodic changes in amplitude of respiration. Furthermore, that the underlying mechanism is principally that of the mechanical effect of changes in intra-thoracic pressure. The experimental work has been limited to the dog, cat and rabbit.

In studying the effects of changing respiratory amplitude upon blood pressure it was imperative that a method be used to obtain records of intra-thoracic pressure changes and it was desirable that these be in graphic form. In the initial experiments the following method was resorted to: a trocar was inserted directly into the thorax of the anestherized animal. This trocar was attached to an inverted bellows by means of a rubber tube provided with a side opening.

The procedure was to clamp off both side tube and tube leading to the trocar. The trocar was then passed between the ribs of the animal. Collodion was then poured around the trocar, thus making an air tight seal. The clamp closing the tube to the trocar was then opened. Since the bellows was held in the folded position initially, no movement would occur

until this tube was opened. The weight of the coverpiece then was often sufficient to open the bellows far enough to allow the recording of the deepest inspiration of the animal. At times it was necessary to admit a small quantity of air by means of the side tube. By experimentation the proper amount of air needed and the proper weight of the coverpiece could be ascertained for the given animal.

Using the foregoing method of recording the respiration, a series of experiments was performed upon rabbits. In each case a simultaneous record of the variations of the intra-thoracic pressure and blood pressure was obtained. After some thirty such experiments had been performed it was found that there were some of the longer blood pressure changes which

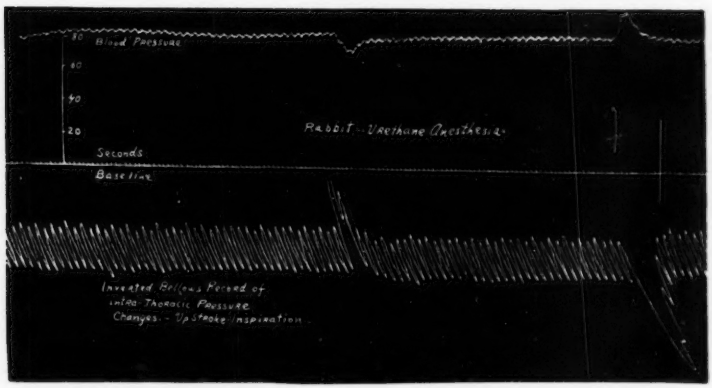

Fig. 1. Simultaneous record of blood pressure and respiration in rabbit under urethane anesthesia, showing spontaneous changes in respiration with concomitant changes in blood pressure. Upstroke = inspiration; downstroke = expiration.

occurred simultaneously with the changes in the intra-thoracic pressure recorded (see fig. 1).

In approaching the problem it was apparent that to study the mechanical effect of a series of respiration upon the blood pressure, that all central nervous influences should be removed; namely, the normal vaso-motor control and other possible reflex effects exerted through the spinal cord. Several experiments were then performed upon spinal cats and rabbits, which were still in the state of shock. By this procedure it was possible to eliminate entirely the possibility of medullary control of the blood pressure. In order to perform the experiments on decapitated cats and rabbits it was necessary to resort to artificial respiration. The respiration was made to conform as much as possible to the normal for the animal. This was accomplished by placing the animal in a respirator similar to that

used by Chillingworth and Hopkins (1) and impressing rhythmic negative pressure variations within the container to simulate normal respiratory movements.

In measuring the amount of intra-thoracic pressure changes graphically, a trocar was passed through the respirator and into the chest of the animal, and records obtained in the same manner as in the preliminary experiments. When, during a given period of observation, the respiration was kept constant in regard to rate and amplitude no blood pressure changes occurred other than those accompanying the individual heart beats and respiratory cycles. However, it was observed that when a single deep inspiration was superimposed upon the constant variation in respiration, an immediate fall in blood pressure was the rule. Or, if a single deep

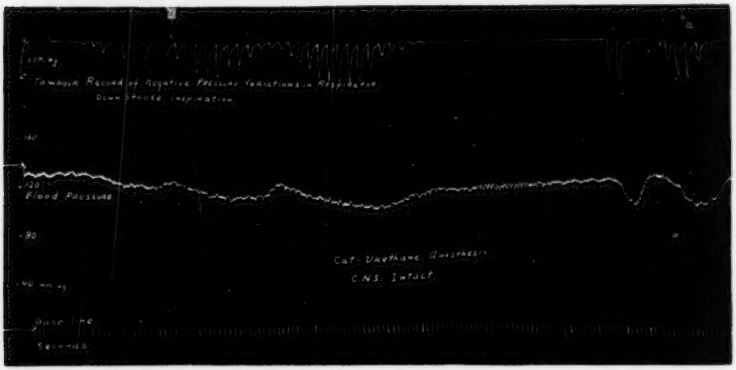

Fig. 2. Simultaneous record of blood pressure and negative pressure changes within respirator of intact cat under urethane anesthesia.

expiration was superimposed upon the constant respiration, an immediate rise in the blood pressure was the rule. Again, when the respiration was modified in amplitude to secure irregular respiration, over a given time interval, the effect upon the blood pressure was that of the production of irregular waves corresponding to the irregular respiration. Or, if the variations of the respirations were so controlled as to produce regular changes in the amplitude of respiration, the effect upon the blood pressure was the production of regular waves in the blood pressure record corresponding to the variations in respiration.

In view of the fact that the spinal cord was intact in this series of experiments, there existed a possibility that spinal reflex control of blood pressure and respiration might be present and play a part in the production of the waves of blood pressure. That the spinal cord of the mammal contains secondary respiratory centers was reported by Swindle (2). In order to

eliminate this possibility, a series of experiments was then performed upon animals (dogs, cats and rabbits) which had been decapitated and the cord pithed. The procedure differed somewhat from the previous in that the

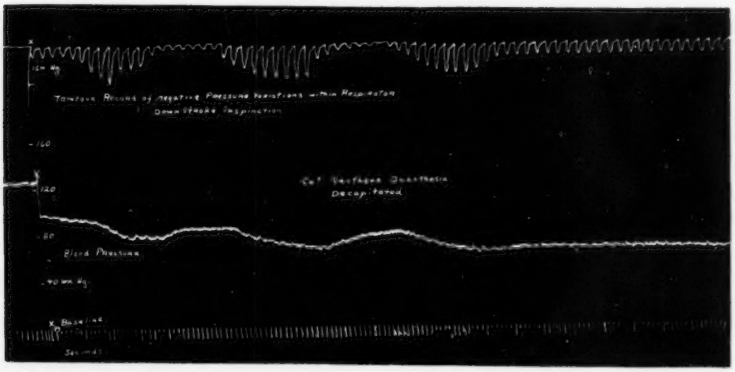

Fig. 3. Simultaneous record of blood pressure and negative pressure changes within respirator on decapitated cat. Downstroke = inspiration; upstroke = expiration.

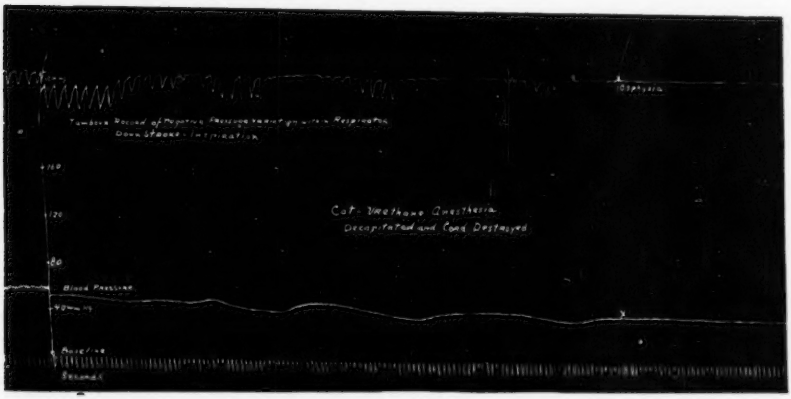

Fig. 4. Simultaneous record of blood pressure and negative pressure changes within respirator on decapitated and pithed cord cat. Downstroke = inspiration; upstroke = expiration.

respirator was only allowed to include the chest proper and extend on the abdomen far enough to include the diaphragm. Intra-thoracic pressure as such was not recorded. The amount of impressed negative pressure within the respirator was graphically recorded with a tambour.

The preliminary procedure in this series was to observe the effect of a series of impressed artificial respiratory movements upon the blood pressure of the intact animal. This procedure in each instance was marked by changes in the blood pressure record which in turn occurred simultaneously with the impressed respiratory movements (see fig. 2).

Decapitation was then performed and by keeping the respiration constant in regard to amplitude, no change in the blood pressure was observed, other than those due to individual heart beats and respiratory cycles. However, when the amplitude was varied within normal limits over a period of time, the changes in blood pressure were marked and corresponded to the changes in the respiration. It was significant to note that after decapitation the blood pressure changes were practically as great as those which were obtained on the intact animal (see fig. 3).

The cord was then pithed and it was apparent that the changes in blood pressure still persisted and corresponded to the changes in the respiratory amplitude (see fig. 4).

Discussion. The records show definitely that changes in depth of respiration have a marked effect upon blood pressure. That the intrathoracic pressure through mechanical virtues may influence the systemic blood pressure can be surmised when the literature is studied concerning the mechanism of production of the respiratory waves of blood pressure. Heinbecker (3) has shown that infiation of the lungs resulted in diminished pulmonary resistance to blood flow and an increased vascular capacity of the lungs. Lewis (4) furnished evidence that the capacity or potential capacity of the lungs is definitely associated with the rapidity of the intrathoracic pressure changes. Daly (5) has shown that a reduction of the mean intra-thoracic pressure is followed by an increase in the total output of the heart, a result of decrease in resistance to blood flow through the lung vessels. Lewis (6) has demonstrated that a fall in the intrapericardial pressure results in a rise in blood pressure. Visscher (7), on the other hand, has concluded that lowered intra-thoracic pressure facilitates the flow of blood to the atria but inhibits the flow of blood through the lungs because of increased rather than decreased pulmonary resistance. Hill, Barnard and Sequeira (8) have shown that an increase in intrathoracic pressure resulted in a lowering of the arterial tension. Heinbecker (3) has also shown that deflation of the lungs increases pulmonary resistance and diminishes pulmonary vessel capacity. Hill and Barnard (9) have demonstrated that positive pressure ventilation resulted in a rise in arterial tension. Chillingworth and Hopkins (10) have called attention to the fact that with prolonged moderate experimental distention of the lungs there occurs a marked lowering of the arterial blood pressure, the amount of lowering depending upon the degree of distention. They came to the conclusion that the fail in blood pressure was due to inadequate

blood supply to the left heart, the result of pressure exerted directly upon the small pulmonary vessels.

From these observations it is clear that in the production of respiratory waves of blood pressure there are a number of factors concerned which are more or less directly the result of changes in intra-thoracic pressure. Most of the investigators agree in that a lowered intra-thoracic pressure results in diminished pulmonary resistance to blood flow, facilitation of flow of blood to the atria, increased vascular capacity of the lungs, increased cardiac output and increased blood pressure. On the other hand, investigators have shown that there results from decreased intra-thoracic pressure an increased pulmonary resistance to blood flow, this resistance acting antagonistically to those changes which facilitate cardiac output.

Our findings are in many respects similar to those observed by Chillingworth and Hopkins, differing only in the manner of production. Our waves apparently are the result of summation of effects, namely, distention within normal limits occuring with changing amplitude of respiration.

Dale and Evans (11) have called attention to the observations that in a spinal cat preparation the blood pressure is depressed by excessive ventilation with pure air, and that subsequently when destruction of the cord was carried out the excessive ventilation brought about a rise in the arterial blood pressure. The explanation for their results was made on the basis that changes in carbon dioxide tension in the blood alternately produced depression and recovery of the vasomotor centers in the bulb and spinal cord.

In our records the waves were produced by normal respiratory changes, in which no attempt was made to alter the carbon dioxide tension of the blood. Under these circumstances the decapitated, cord pithed cat reacted to the changing respiration in a similar manner to that of the decapitated and intact animal preparations. Furthermore, when the decapitated, cord pithed animal was asphyxiated no change occurred in the blood pressure, thus suggesting that the changes in intra-thoracic pressure may act mechanically in the production of the waves since the spinal vasomotor centers had been removed.

SUMMARY AND CONCLUSIONS

1. It is possible to produce experimentally waves of blood pressure in intact, decapitated and cordless animals by changing the respiratory amplitude within normal limits.

2. The resultant changes in blood pressure are most likely due to the mechanical effects of changing intra-thoracic pressure.

3. The evidence obtained shows that some of the longer regular or irregular waves of blood pressure which are characterized by a relatively rapid fall in blood pressure with a relatively rapid return to normal, may

have as their origin the changes occurring in intra-thoracic pressure, and need not be interpreted as being due to changes in vasomotor tone.

I am indebted to Dr. A. E. Guenther and Dr. Charles C. Johnson for their timely advice and suggestions.

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THE EFFECT OF VIOSTEROL ON THE CALCIUM CONTENT OF DOG'S BILE[1]

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It is well known that calcium is a constant constituent of the bile (1). Since calcium is concerned in gall-stone formation, an increase in the calcium of the bile might predispose to gall-stone formation, particularly since Dittrich (2) has shown that normal ox bile is very readily saturated with a small amount of calcium (14–17 mgm. per 100 cc.). It is also well known that viosterol in large doses will increase the blood calcium level (3) (4), but the effect of viosterol on biliary calcium and bile output is not known. Since relatively large doses of viosterol are now given in many clinical conditions and particularly in pregnancy where the incidence of gall-stone formation is high, we have undertaken to ascertain whether this use of viosterol would lead to an increased output of calcium in the bile.

EXPERIMENTAL PROCEDURE. In this work twenty-five dogs were used. Of these, twelve were operated according to the technique of McMaster, Brown and Rous (5), in which after cholecystectomy the common bile duct is cannulated with a glass cannula to which is attached a rubber tube which is looped in the abdominal cavity and then brought through the abdominal wall and attached to a sterile rubber balloon. In our work, the balloon filled with bile was detached and replaced by a sterile fresh balloon each day.

The dogs operated in this way had a tendency to slough their cannnulae and to reëstablish the common bile duct after twenty or more days. To avoid this difficulty, the common bile duct in thirteen of the dogs was doubly ligated, cut and a section of the duct removed, the cannula being placed in the cystic duct.

These dogs were kept on the stock diet to which they were accustomed but in addition, because their appetite was variable, a mixture of mucin, milk, glucose, sodium bicarbonate, and calcium carbonate was given by stomach tube. This mixture had the following composition: mucin, 15 grams; sodium chloride, 4 grams; sodium bicarbonate, 4 grams; calcium carbonate, 4 grams; corn syrup, 15 cc.; and milk, 150 cc. This mixture

[1] This work was aided by a grant from the Mead Johnson and Co.

was given to provide alkali to replace that lost in the bile, and to provide an excess of calcium for absorption. In addition the bile, after being mixed thoroughly, measured and sampled for analysis, was added to the food and salt mixture and returned to the dog.

The calcium in the bile was determined according to a method which is essentially that of Drury (1) in which the bile is ashed in platinum, the calcium precipitated as the oxalate, and the oxalic acid of the calcium oxalate titrated with N/100 potassium permanganate.

The calcium in the blood was determined by the Clark-Collip (6) modification of the Kramer-Tisdall method as it is commonly used.

The viosterol used in this experiment was of two concentrations, either the 250 D, which is the standard viosterol of commerce, or the 10,000 × which is 100 times as concentrated as the 250 D. In some experiments, the

TABLE 1

Distribution of 193 bile analyses

NO VIOSTEROL

Concentration of Ca mgm./100 cc. bile	23	22	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5
Number of analyses at each concentration	1	4	1	2	3	3	10	11	27	37	32	34	7	10	1	2	7	0	1

Distribution of 73 bile analyses

AFTER VIOSTEROL (5-10 CC. 250D DAILY)

Concentration of Ca mgm./100 cc. bile	20	20	19	18	17	16	15	14	13	12	11	10	Below 10
Number of analyses at each concentration	3	1	1	2	7	5	15	13	8	10	3	2	2

viosterol was mixed with the food. Where the food was not consumed readily, the viosterol was given by stomach tube, usually with the bile-milk-mucin mixture. In three dogs the viosterol 10,000 × was given intravenously for three or four days in succession. This was done by removing some of the dog's blood in a sterile tube, removing the serum after centrifuging, and using this serum as an emulsifying medium in which state the finely divided viosterol was injected.

EXPERIMENTAL RESULTS. *Administration of 250 D in large doses.* After suitable control studies were made, a dose of from 5 to 10 cc. of 250 D Viosterol was given daily. This dose is from four to eight times that ordinarily recommended as the maximum therapeutic dose for man. As expected from reports in the literature, the volume of the bile and the bile calcium analyses varied from day to day with minor fluctuations.

In view of this fact and also because of the rather large number of analyses obtained, it is believed that a statistical treatment of the data would give the best interpretation. The data in table 1 show the distribution of 193 analyses of bile calcium before the administration of viosterol and of 73 analyses after the oral administration of from 5 to 10 cc. of 250 D.

By treating these data statistically, it is found that the mean analysis of the normal bile is 13.64 ± 0.14 with a standard deviation of 2.90 ± 0.10,

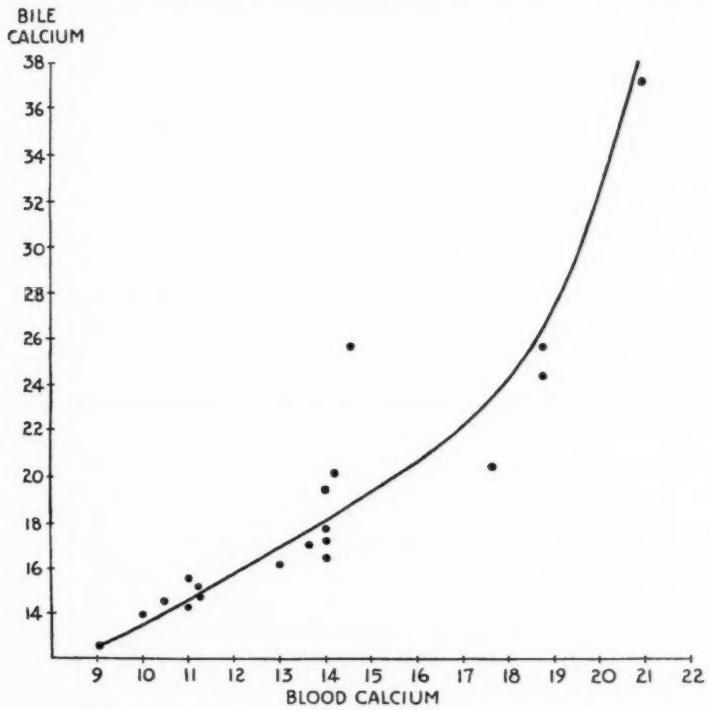

Fig. 1

which indicates a narrow deviation from the mean of the various analyses. The mean of the analyses after the administration of viosterol in large therapeutic doses, such as 5 to 10 cc. of 250 D per day is 14.44 ± 0.13 with a standard deviation of 2.57 ± 0.09. The difference then between the amount of calcium in the bile before and after viosterol administration is, therefore, the difference between the lower limit of the mean after viosterol and the upper limit of the mean calcium before viosterol was administered. Thus, (14.44 − 0.13) − (13.65 + 0.14) = 0.53, the difference of 0.53 mgm. per 100 cc. of bile is essentially negligible.

The blood calcium and bile output were not significantly altered by the doses of 250 D used in these experiments.

Administration of 10,000 × units of D intravenously. The intravenous injection of 1 cc. of 10,000 × viosterol, however, raises the blood calcium

TABLE 2

Showing the correlation between the concentration of calcium and bile pigment in hepatic bile

	VARIOUS SAMPLES														
Bile pigment mgm./100 cc.	655	443	440	392	332	324	318	295	282	272	212	170	168	121	115
Bile calcium mgm./100 cc.	19.4	14.5	9.4	12.8	11.8	14.7	15.7	16.6	17.4	11.5	15.3	11.0	11.3	10.4	12.4

TABLE 3

Distribution of the data for the computation of the correlation coefficient of the daily fluid volume output of bile to the total calcium output in the bile

TOTAL OUTPUT OF CALCIUM IN MGM.	VOLUMES OF BILE IN CC.																			DISTRIBUTION OF TOTAL CALCIUM OUTPUT
	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	
2	2																				2
4	3	11	4		1																19
6			6	8		1		2													17
8		1	2	3	7	2	5	1			1	1									23
10				2	5	4	10	3													24
12							3	8	3	3	2										19
14							2	6	10	11	1	2									32
16						1			4	5	1	5	1	2	1	1					21
18										1	4	1	2	2				1			11
20						1			2		3		3	4	5	1	1			1	21
22														2	2		1				5
24														1		1		1			3
26															1	1	2		1		5
28																			1		1
30																		1			1
Distribution of volume output...	5	12	12	13	13	9	20	20	19	20	12	9	6	11	9	4	4	3	2	1	204

Mean fluid volume output = 86.18 cc. ± 2.06.
Mean total calcium ouput = 12.68 mgm. ± 0.28.
Standard deviation of fluid volume output = 43.64 ± 1.46.
Standard deviation of total calcium output = 5.86 ± 0.20.
Coefficient of correlation 0.906 ± 0.004.
Line of regression $y = 0.608\,x$.

and at the same time the bile calcium increases. This relationship is plotted in figure 1.

It is evident from the data in figure 1 that the calcium concentration rises

in the bile at a faster rate than it rises in the blood which is correlated with a very definite decrease in the bile volume output, which occurs when the blood calcium is high. In this case much semi-solid matter comes out with the bile, which forms a sediment rapidly on standing.

The volume output of bile was definitely decreased when the blood calcium reached 15 mgm. per 100 cc., the output being practically abolished when the blood calcium was between 20 and 25 mgm.

Bilirubin and bile calcium. Since bilirubin is an acid and combines with bases and alkaline earth metals, it is possible that the calcium in the bile may depend on the bilirubin excreted. There does not appear to be any correlation, however, between the pigment and the calcium output, although both tend to be high following operation. Data in table 2 are arranged to show this lack of correlation which again points out the differential activity that the liver manifests in the performance of its many functions.

Relation of total calcium output to fluid volume of bile. Drury (1) has indicated that the total amount of calcium excreted in the bile is dependent on the volume of the bile, since the concentration tends to remain constant. Since the volume of the bile was measured in these experiments, an opportunity is provided to determine a numerical figure for this relationship. The correlation coefficient is found to be 0.906 ± 0.004—a very high degree of correlation. The distribution of the data for the computation of this figure is given in table 3. From this may be obtained the equation of this relationship, the line of regression of which is $y = 0.608\,x$.

Discussion. The data obtained in these various experiments indicate that the calcium of the bile is dependent on the calcium content of the blood, and that the bile is one of the routes for the excretion of the excess blood calcium. However, the calcium content of the hepatic bile is always higher than that of the blood. This higher level of 1 or 2 mgm. per cent over that of the blood calcium may be due to a slight concentration of the bile in the intra and extrahepatic ducts. It can hardly be due to concentration by evaporation from the balloon during the day, since a thick-walled balloon was used and covered by a metal pan strapped close to the body of the dog.

If these data on the dog can be carried over to man, it is quite obvious that the administration of viosterol to the human in the therapeutic doses recommended and commonly employed does not influence the calcium concentration of the bile. If, however, sufficient viosterol is administered to elevate definitely the blood calcium, an increase in bile calcium will probably result.[2]

[2] Although we desired to confirm this work on a human patient with a chronic biliary fistula, such a patient was not available to us during the course of this study.

CONCLUSION

1. From these experiments it is evident that large doses of viosterol, larger than would be ordinarily used in clinical practice, do not raise the bile calcium concentration, or modify bile output, in the dog.

2. It is shown that doses large enough to increase definitely the blood calcium level will increase the bile calcium concentration and decrease the output of bile.

3. The data show that the concentration of the calcium in the hepatic bile of the dog is quite uniform when the bile flow and blood calcium level are normal and is numerically equal to 13.64 $\pm$ 0.14 mgm. per 100 ml. of bile.

4. A correlation of the calcium excreted in the bile to the volume of the bile shows a high coefficient of 0.90 $\pm$ 0.004, which shows that under normal conditions the total calcium excreted by way of the bile is almost wholly dependent on the volume output.

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THE RESPIRATORY METABOLISM OF INFRAHUMAN PRIMATES[1]

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During the last decade the infrahuman primates have come to be used extensively as experimental animals, but no attempt has previously been made to compare their basal metabolic rates. Since the autumn of 1931, however, the heat production of the rhesus moneky has been under study by the Nutrition Laboratory in the Department of Embryology of the Carnegie Institute of Washington at Baltimore, and this work is being continued at the present time. Also, Botschkareff has published preliminary studies on the respiratory exchange of one adult rhesus monkey and three mandrills.

Animals used and procedures. Our experiments include observations on monkeys, baboons, a gibbon, an orang outan, and chimpanzees. In all, 17 animals (classified according to Flower, see Zuckerman and Fulton, 7), were used in the experiments, the species being as follows:

Monkeys:	
Macaca Mulatta	6 specimens
Cercocebus torquatus lunulatus	3 specimens
Baboons:	
Papio papio	3 specimens
Gibbon:	
Hylobates lar	1 specimen
Orang outan:	
Pongo pygmaeus	1 specimen
Chimpanzees:	
Pan satyrus	3 specimens

Preliminary observations on the activity of monkeys in the metabolism chamber made it clear that experiments could not be performed during the day, even though the chamber were darkened. Accordingly, observations were carried out at night when the animal was normally asleep and when

[1] The data in this paper are taken from a dissertation presented in June, 1933, to the Graduate School of Yale University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. (A preliminary note concerning these studies was published under the above title in THIS JOURNAL 1934, **109**: 16, 1934. Proceedings.)

there were fewer disturbing noises. All observations reported have thus been obtained on the sleeping animal between the hours of 9:00 p.m. and 3:00 a.m. The animal to be used in the evening was fed early the preceding morning after which time food was denied for the rest of the day; therefore, the subject was at least 12 hours, and not over 18 hours, post-absorptive.

The apparatus for determining the respiratory exchange consisted of a water jacketed chamber of suitable size and a Benedict Universal Respiration Apparatus. A cage built to the dimensions of the chamber confined the animal during experimentation and also served as a transfer cage from living quarters to experimental room. In the chamber, this cage rested on a crude knife edge at one end and at the other end on a system of springs, rubber tubing, tambour and smoked drum for recording the subject's motility. Observations were made during 30-minute periods.

Alcohol was frequently burned in the chamber and its respiratory quotient determined. A respiratory quotient of 0.65 to 0.68 was taken as evidence of satisfactory functioning of the apparatus.

Surface area and its determination in the apes. It seemed desirable to express the heat production not only per unit of weight but also per unit of surface area according to current custom. The surface area of the monkey and the baboon was obtained by using the Meeh (10) formula, $S = KW^{2/3}$. The value for K of 11.7 has been determined by Lee and Fox (9) for the rhesus monkey. The difference in shape and the absence of a tail in the anthropoid apes and the difference in weight between, for example, the gibbon and the chimpanzee, made it desirable to measure directly the surface area of these animals. The method used, that of DuBois and DuBois (6), consisted of determining the surface area of a snugly fitting, inelastic but removable cast. Since the subject must be immobile during the fitting of the cast, the animal was placed under amytal anesthesia. Gauze was then wrapped about the body to which gummed paper was rapidly affixed. The resulting cast was removed by bandage scissors, painted on the inside with hot paraffin, and when cool, cut into irregular pieces which would lie flat upon photographic paper. The cast was then photographed and the unexposed portions of the paper were cut out and weighed. The surface area was obtained as a simple proportion between the weight of the unexposed paper and the weight of the original sheet of paper to the area of the unexposed paper and the surface of the original sheet.

The surface areas of the gibbon, orang outan, and two chimpanzees were measured in this manner. For the orang outan, weighing 16.2 kgm., the value of K was found to be 11.3; for the chimpanzees, weighing 14.1 and 24.5 kgm., the values were 11.0 and 10.5 respectively. As might be expected, the value of K for the gibbon, an animal with a thin trunk and

TABLE 1

The respiratory metabolism of normal animals

ANIMAL	SEX	AGE	WEIGHT	O_2/HR: I.	R.Q.	CAL./KILO 24 HRS.	CAL./ SQ.M. 24 HRS.	CHAMBER TEMPERATURE
Rhesus	M.	A.	3.70	1.70	0.81	53.1	701	22.5
Rhesus	F.	A.	4.35	1.71	0.84	45.8	638	23.8
Rhesus	F.	A.	8.10	2.56	0.78	36.2	621	23.3
Rhesus	F.	A.	8.10	2.68	0.78	37.9	650	23.2
				Average for rhesus			653	
Mangabey	M.	I.	3.40	1.49	0.80	50.5	649	22.7
Mangabey	M.	I.	3.40	1.47	0.85	50.5	649	23.8
Mangabey	M.	I.	3.40	1.48	0.79	50.0	642	24.0
Mangabey	M.	I.	4.10	1.69	0.81	47.6	650	23.0
Mangabey	M.	I.	4.10	1.72	0.77	48.0	657	24.0
Mangabey	M.	I.	4.10	1.75	0.80	49.2	673	23.3
				Average for mangabey			653	
Baboon	M.	A.	5.30	2.45	0.78	53.0	789	23.3
Baboon	M.	A.	5.80	2.53	0.77	49.7	762	23.4
Baboon	M.	A.	5.80	2.54	0.78	50.2	770	23.5
Baboon	M.	A.	7.60	2.91	0.76	43.7	735	22.5
Baboon	M.	A.	7.60	2.99	0.79	45.2	761	22.4
Baboon	M.	A.	7.60	2.74	0.75	41.0	690	24.3
Baboon	M.	A.	7.60	2.88	0.75	43.1	728	23.8
				Average for baboon			748	
Gibbon	M.	I.	1.90	1.15	0.79	69.6	658	23.4
Gibbon	M.	I.	1.90	1.02	0.79	61.7	583	24.5
Gibbon	M.	I.	1.90	1.05	0.79	63.5	600	25.7
				Average for gibbon			613	
Orang outan	F.	A.	16.20	5.01	0.77	35.4	793	22.9
Orang outan	F.	A.	16.20	4.98	0.78	35.2	791	23.2
Orang outan	F.	A.	16.20	4.92	0.78	34.8	781	24.2
				Average for orang outan			788	
Chimpanzee	M.	A.	14.1	3.90	0.82	32.0	700	23.0
Chimpanzee	M.	A.	14.1	4.28	0.79	34.9	763	22.9
Chimpanzee	M.	A.	14.1	4.18	0.83	34.4	742	23.1
Chimpanzee	M.	A.	16.4	4.53	0.80	31.8	749	22.9
Chimpanzee	M.	A.	16.4	4.73	0.75	32.8	772	23.5
Chimpanzee	M.	A.	16.4	5.19	0.78	36.3	854	23.3
Chimpanzee	M.	A.	16.4	4.66	0.79	32.7	768	22.8
Chimpanzee	M.	A.	24.5	6.42	0.80	30.2	834	25.5
Chimpanzee	M.	A.	24.5	6.16	0.79	28.9	798	23.6
Chimpanzee	M.	A.	24.5	6.15	0.85	29 3	809	23.5
				Average for chimpanzee			779	

long, slender limbs, weighing 1.9 kgm., was appreciably higher than the values for either the orang outan or chimpanzee, 13.0.

RESULTS. *1. Heat production.* The data concerning the weight of animal, its respiratory quotient, O_2 consumption, heat production per unit of weight and per unit of surface area are collected in table 1. It was found that both the adolescent rhesus and the immature mangabey monkey when studied at an environmental temperature of approximately 23°C. gave an average metabolic rate of 653 calories per square meter in 24 hours. The similarity of the metabolic rates of the rhesus and the mangabey monkey is still further emphasized by comparison of the heat production per unit of weight of a rhesus weighing 4.35 kgm. and mangabey weighing 4.1 kgm. The former animal produced 46 calories and the latter 48 calories per kilogram of body weight.

The heat production of the group of 3 adolescent baboons was greater than that of the monkeys—748 calories per unit of surface area for the baboons as compared to 653 calories for the monkeys. Comparison of heat production per unit of weight in a baboon, 7.6 kgm., and in a rhesus monkey, 8.1 kgm., showed a metabolic rate some 16 per cent higher for the baboon. It is perhaps better at the present time to accept with caution the statement that the baboon has a higher metabolic rate than the monkey, since all the baboons were males but only one of the three rhesus monkeys was a male. Subsequent studies on large groups of males and females may show, as Benedict and Talbot (3) have shown for the human, that the females have a lower basal energy production than the males. A sex difference of 7 to 10 per cent would leave the difference insignificant between the rhesus monkeys and the baboons used in this experiment. Since the mangabey monkeys were all males and immature, one might argue, however, that they would have a metabolic rate higher than that which might be expected for the adolescent females.

The immature gibbon was characterized by the low rate of heat production of 613 calories per square meter of surface area in 24 hours. With the larger anthropoids, however, the rates were higher. The orang outan and the chimpanzees are quite similar in heat production both per kilogram and per square meter of body surface. The average heat production of the three chimpanzees is 779 calories per square meter of body surface as compared to 788 calories for the orang outan, and 33.4 and 35.1 calories per kilogram respectively for chimpanzee and orang outan of almost identical weights. It must be remembered, however, that the orang outan was a female and all of the chimpanzees were males.

From these experiments it is clear that two species of the great apes, the orang outan and chimpanzee, have a higher metabolic rate than the rhesus and mangabey monkey but a rate insignificantly different from the baboon. One young gibbon manifested a lower rate than found for any of the animals.

TABLE 2

The respiratory metabolism of operated animals

ANIMAL	LESION	SEX	AGE	WEIGHT	O_2/HR. L.	R.Q.	CAL./KILO 24 HRS.	CAL./SQ.M. 24 HRS.	CHAMBER TEMPERATURE
Mangabey	Normal	M.	I.	3.40	1.49	0.80	50.5	649	22.7
Mangabey	Normal	M.	I.	3.40	1.47	0.85	50.5	649	23.8
Mangabey	Normal	M.	I.	3.40	1.48	0.79	50.0	642	24.0
						Average		647	
Mangabey	Unilateral	M.	I.	3.50	1.45	0.77	47.4	614	22.5
Mangabey	Unilateral	M.	I.	3.50	1.45	0.76	47.3	613	23.6
						Average		614	
Mangabey	Bilateral	M.	I.	3.40	1.49	0.74	49.7	638	22.9
Mangabey	Bilateral	M.	I.	3.40	1.46	0.76	49.0	628	23.2
						Average		633	
Mangabey	Normal	M.	I.	4.10	1.69	0.81	47.6	650	23.0
Mangabey	Normal	M.	I.	4.10	1.72	0.77	48.0	657	24.0
Mangabey	Normal	M.	I.	4.10	1.75	0.80	49.2	673	23.3
						Average		660	
Mangabey	Unilateral	M.	I.	4.00	1.64	0.78	47.0	637	23.5
Mangabey	Unilateral	M.	I.	4.00	1.69	0.77	48.3	655	23.2
Mangabey	Unilateral	M.	I.	4.00	1.67	0.79	48.0	651	23.4
						Average		648	
Mangabey	Bilateral	M.	I.	4.05	1.69	0.79	48.0	654	22.5
Mangabey	Bilateral	M.	I.	4.05	1.70	0.80	48.4	660	23.0
Mangabey	Bilateral	M.	I.	4.05	1.73	0.82	49.5	674	22.8
Mangabey	Bilateral	M.	I.	4.05	1.67	0.77	47.1	643	22.9
Mangabey	Bilateral	M.	I.	4.05	1.65	0.77	46.6	635	23.2
						Average		653	
Mangabey	Unilateral	M.	I.	3.80	1.63	0.77	49.1	654	23.0
Mangabey	Unilateral	M.	I.	3.80	1.44	0.75	43.1	575	23.5
Mangabey	Bilateral	M.	I.	4.00	1.69	0.85	49.3	668	22.8
Mangabey	Bilateral	M.	I.	4.00	1.75	0.75	49.7	675	23.8
Mangabey	Bilateral	M.	I.	4.00	1.69	0.75	48.1	652	23.0
Mangabey	Bilateral	M.	I.	4.00	1.67	0.75	47.5	644	25.1
Mangabey	Bilateral	M.	I.	3.80	1.59	0.88	49.2	656	24.0
						Average		659	
Rhesus	Bilateral	M.	I.	3.7	1.65	0.75	50.7	670	22.6
Rhesus	Bilateral	M.	I.	3.7	1.63	0.77	50.4	666	23.4
						Average		668	

Explanation of symbols: A = adolescent; I = immature; M = mature.

TABLE 2—*Concluded*

ANIMAL	LESION	SEX	AGE	WEIGHT	O_2/HR. L.	R.Q.	CAL./ KILO 24 HRS.	CAL./ SQ.M. 24 HRS.	CHAMBER TEMPERATURE
Rhesus	Hypothalamic	M.	I.	5.75	2.09	0.80	41.9	640	23.2
Rhesus	Hypothalamic	M.	I.	5.80	1.93	0.79	38.2	587	22.5
Rhesus	Hypothalamic	M.	I.	5.80	1.78	0.80	35.4	543	23.1
Rhesus	Hypothalamic	M.	I.	5.80	1.80	0.75	35.3	542	23.3
Rhesus	Hypothalamic	M.	I.	5.80	1.87	0.80	37.2	570	23.0
Rhesus	Hypothalamic	M.	I.	5.80	1.83	0.81	36.4	559	21.9
						Average		574	
Rhesus	Oophorectomy	F.	M.	6.5	2.29	0.80	40.6	647	23.6
Rhesus	Oophorectomy	F.	M.	6.5	2.15	0.79	38.0	606	25.3
Rhesus	Oophorectomy	F.	M.	8.0	2.46	0.76	35.1	600	23.2
Rhesus	Oophorectomy	F.	M.	8.0	2.21	0.75	31.4	537	23.7
Rhesus	Oophorectomy	F.	M.	8.0	2.18	0.76	31.1	531	25.0
						Average		584	

Botschkareff's (5) figures for the metabolic rate of monkeys were obtained, as he has stated, under such conditions that they can not be taken as basal. In his experiments a mask was fitted to the animal's face and the animal was placed in a bag to be held on the lap of an assistant during the collection of gas. Under these conditions, the animal often struggled but occasionally appeared to be asleep. The results vary from 293 calories for one animal to 15,000 calories for another animal weighing 5 kilograms. The results are obviously not comparable with our results.

2. *Respiratory quotient.* The respiratory quotients which have been obtained on these animals indicate that they come to a fasting level as quickly, if not more quickly, than human subjects. The human respiratory quotient, 12 hours after the last meal, is usually taken to be 0.82, while the respiratory quotient as determined on these animals 12 to 18 hours after the last meal averages 0.79. Botschkareff found values of 0.83, 0.88, 0.82 and 0.82 (averages for each animal for the four monkeys he used). They were reported to be at least 12 hours post-absorptive.

Discussion. A comparison of the results on these primates with the results which have been obtained on humans or on other mammals, although interesting, would be premature at this time. Obviously, the special requirements imposed by the necessity of controlling activity permitted use of the animal only at night when asleep. In the human, sleep is known to have a depressing effect of about 10 per cent upon the basal metabolic rate (2). Due principally to the rather small number of available animals, it has been impossible to obtain data concerning certain factors, specifically age and sex, which are known to exert some effect on the

basal metabolic rate. However, it is clearly significant that higher anthropoid apes occupy a position intermediate between man and monkey with regard to their basal metabolic rates.

As evidence of the adequacy of our methods and conditions of measurement, the following instances of metabolic determinations, made after injury to the nervous system and after removal of the ovaries, may be cited.

1. *Absence of change in basal metabolic rate following removal of frontal areas.* In a study of the relation of the frontal lobes to posture and forced grasping in monkeys, Fulton, Jacobsen and Kennard (8) observed that following removal of the frontal areas there is an augmentation of appetite which sometimes causes the animal to ingest two or three times the normal amount of food; yet despite this circumstance the animals generally lose weight. The question arose whether the increase of appetite was associated with a disturbance of metabolism which might be reflected in the metabolic rate. Two mangabey monkeys were therefore studied before operative procedure, and again after unilateral, and after bilateral removal of the frontal areas. Another mangabey and one rhesus were studied after bilateral removal of the frontal areas. The normal animals gave average values of 646 and 660 calories per square meter in 24 hours, but after unilateral removal, 614 calories and 648 calories, and after bilateral removal, 633 and 653 calories. Since the determinations obtained on the third monkey after unilateral removal of the frontal cortex varied greatly, they were disregarded. The average of five determinations after bilateral removal of the frontal cortex was 659 calories, a heat production close to that observed for the normal animals and for the two animals after bilateral removal of the frontal cortex. The rhesus monkey produced 668 calories after operation as compared to 653 calories of the normal rhesus monkeys. It is concluded, therefore, that removal of the frontal cortex had no observable effect on the metabolic rate.

2. *Changes after other procedures.* One rhesus monkey following a hypothalamic lesion gained 2 kgm. of weight in one month and at autopsy 6 months after the operation showed marked deposits of fat about the shoulder girdle, in the anterior body wall, and in the greater omentum. An average heat production of 574 calories was observed for this animal after operation as compared to 653 calories for the normal rhesus monkeys. Two other rhesus monkeys from which the ovaries had been removed, averaged 592 calories as compared to 653 calories for the normal animal.

SUMMARY

The respiratory metabolism of various infrahuman primates has been studied using the Benedict Universal Respiration Apparatus and a chamber.

The surface area of the rhesus and mangabey monkeys and the baboons was estimated by the Meeh formula, $S = KW^{2/3}$, in which the constant K is equal to 11.7, as proposed by Lee and Fox for the rhesus monkey. The surface area of the gibbon, orang outan and two of the chimpanzees was measured directly by the method of DuBois and DuBois.

The heat production of the rhesus and mangabey monkeys was approximately 653 calories per square meter in 24 hours for both species.

The baboons had a higher rate of heat production than the monkeys, i.e., 748 calories.

The gibbon averaged only 613 calories per unit of surface per 24 hours.

The orang outan and the three chimpanzees averaged 788 and 779 calories respectively.

The average of respiratory quotients obtained from these animals 12 to 18 hours post-absorptive was 0.79.

Extirpation of the frontal cortex, although augmenting activity, did not alter the metabolic rate. A lesion of the hypothalamus in one animal and removal of the ovaries in two subjects lowered the rate of heat production.

I am much indebted to Dr. Francis G. Benedict of the Carnegie Nutrition Laboratory, who has advised me from time to time in the course of this work, and who, on two occasions, extended to me the privileges of his laboratory. The investigations are now being continued at The Anthropoid Experiment Station, Orange Park, Florida, under the supervision of Doctors Benedict and Robert M. Yerkes.

I am also much indebted to Mr. Delafield DuBois of the department of Physiology, Yale University School of Medicine, for his kind assistance and instruction in measuring the surface area of these animals.

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BOUND WATER IN CARDIAC MUSCLE IN RELATION TO VENTRICULAR FIBRILLATION

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The idea, originally advanced by Balcar, Sansum and Woodyatt (1) that water exists in a "bound" as well as in a "free" state in the cellular structure has aroused considerable interest in recent years as an explanation of certain of the processes in the living cell. Although this concept has been questioned on experimental grounds by A. V. Hill (2) and Grollman (3), a number of other workers accept it as demonstrable, i.e., Rubner (4), Thoenes (5), Meyer (6), Newton (7), Robinson (8), St. John (9) and Sayre (10).

The present paper deals with the question of "bound" water in mammalian cardiac muscle and specifically with the relation of such "bound" water to the condition of ventricular fibrillation.

We used the calorimetric method as elaborated by Sayre and are indebted to him for instruction and advice in its use. This method, for the details of which reference must be made to Sayre's paper (l.c.) depends upon the determination of a number of factors for introduction into an algebraic equation the solution of which gives the amount of "free" or freezable water in the specimen of tissue under investigation. The total water having been determined by desiccation, the difference between the total and "free" water gives the "bound" water.

For our problem the following factors were determined:

a. Correction for calorimeter = 1.0772.
b. Specific heat of dry cardiac muscle = 1.11.
c. Freezing point of cardiac muscle; normal = −0.580°C. and fibrillating = −0.860°C.[1]
d. Fall in calorimeter temperature caused by introduction of frozen tissue.

The technical procedure was as follows. The dog heart was isolated and perfused with Locke's solution in the usual manner. After fifteen minutes of such perfusion approximately 10 grams of ventricular tissue were excised.

[1] These values were obtained with the needle-thermo-couple devised by Karrer and Eastabrook (11), to whom we are greatly indebted for assistance. The method has the advantage of producing minimum injury to tissue structure.

The ventricles were then thrown into fibrillation by faradic stimulation and after five minutes a second corresponding sample was excised.

These two samples were blotted dry, weighed and placed in stoppered freezing tubes. The tubes were placed in a bath containing solid carbon dioxide and ether and maintained at a temperature of $-30° \pm 0.2°C.$ for two hours to accomplish the freezing of the "free" water. The tubes were then quickly and separately transferred to the calorimeter containing 500 cc. water at known temperature. After corking, the contained water was gently stirred until the temperature became constant, when the thermometer was read and recorded. Preliminary tests made it possible to adjust the initial temperature of the water so that the fall on either side of room temperature was about equal, thus minimizing the loss of heat to the outside. This procedure together with the efficient transfer of the tubes to the calorimeter were important factors in obtaining uniform results.

Total water in the muscle sample was obtained by drying the tissue at 100°C. to constant weight.

RESULTS AND DISCUSSION. The average results for six successfully completed experiments were:

	PER CENT TOTAL WATER	PER CENT FREE WATER	PER CENT BOUND WATER
Before fibrillation	80.28	62.26	18.02
After fibrillation	81.30	66.92	14.38

All of the experiments agreed in showing the same trend in the partition of the water but the magnitude of the trend was different with the different hearts.

While the conditions imposed were not strictly normal in that we dealt with artificially perfused tissue the results do indicate a basic change as regards the fibrillation and prefibrillation periods. The percentage increase of total water (1 per cent) is slight and is, presumably, a consequence of on-coming edema. The distribution of water as between the "free" and "bound" state (4.5 per cent) is, however, indicative of a significant change in the functional structure of the cellular tissue. This significance is the more apparent in view of the fact that fibrillation is a reversible condition (12).

The authors express their appreciation to Dr. J. S. Sayre for his assistance at the Agricultural Experiment Station in Wooster, Ohio for practical help in the operation of the method which he has developed for the investigation of the present problem. We wish also to express appreciation to Doctors Karrer and Eastabrook for the use of their apparatus and for assistance in its operation.

SUMMARY

The methods used for the determination of "free" and "bound" water in the ventricular muscle of the dog heart are presented. These methods were applied to specimens obtained from the isolated and perfused heart before and after establishing ventricular fibrillation.

Results are reported to show that in this condition there is an increase of "free" and a decrease of "bound" water in the muscle tissue when the ventricles of the heart are fibrillated.

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EFFECT OF "CHRONIC" EXPERIMENTAL LIVER DAMAGE ON THE BLOOD SUGAR RESPONSE TO INSULIN

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Several reports in the literature, reviewed by Bordley (1), of the disappearance of diabetes mellitus during the development of cirrhosis of the liver, as well as reports by Nadler and Wolfer (2), Crawford (3), and others of marked hypoglycemia in cases of extensive primary carcinoma of the liver, suggest that hepatic damage might result in an increased sensitivity to insulin (see Althausen, ref. 4). Forsgren (5) has also suggested that the glycogen content of the liver undergoes rhythmic changes independent of alimentation, and that the susceptibility of the organism to insulin is greater when the glycogen stores are reduced.

We have tested the effect of liver damage on insulin sensitivity by following the blood sugar response of normal and Eck fistula dogs (prepared by Dr. L. A. Crandall) to small intravenous doses of insulin, according to the method of de Takats and Cuthbert (6). The animals were fasted 16 hours and insulin was given in doses of 0.1 unit per kilogram dissolved in about 1 cc. of normal salt solution. Blood sugars were determined by the Folin modification of the Folin-Wu method before, and 10, 20, 30, 60, 90 and 120 minutes after, the injection. One such test was done on each of 10 normal dogs, and 2 on each of 5 Eck fistula dogs.

The Eck fistula dogs had approximately the same average fasting blood sugar as the normal dogs. It is quite possible, however, that a longer fast would bring out a difference between the two. The average maximum depression of the blood sugar by insulin was 46.0 mgm. per 100 cc. in the Eck group, as contrasted to 35.2 mgm. in the normal group. That this difference of 10.8 mgm. is statistically significant is shown by the fact that the probable error of the blood sugar depression values for the 2 groups is only ± 1.7 and ± 2.0 mgm. respectively. There is no essential difference in the results after applying corrections for the error inherent in the blood sugar method at low values.

Whether the increased insulin sensitivity in this form of hepatic damage is due to diminished liver glycogen stores, to an abnormal fixation of a normal amount of glycogen in the liver, or to impairment of the gluconeo-

genetic mechanism, we cannot say. Probably the first explanation is the correct one, but apparently glycogen determinations have never been made on the livers of Eck fistula dogs. A more remote possibility is suggested by the work of Arborelius and Åkerrén (7), who concluded that insulin introduced through the visceral peritoneum or directly into the portal vein was less active than equal amounts administered by other routes. This, however, has been denied by Aubertin and Trinquier (8).

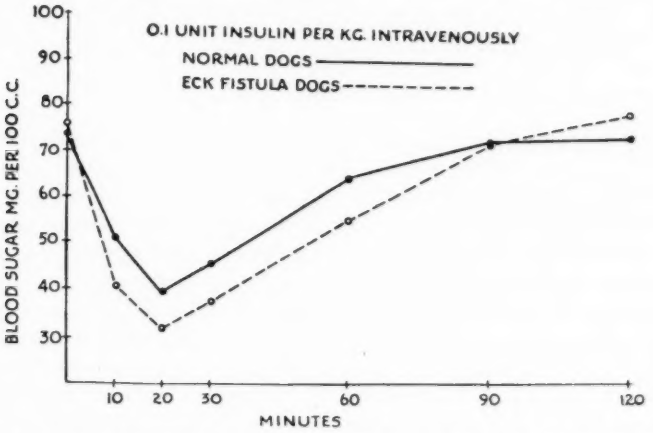

Fig. 1. Average blood sugar response of 10 normal dogs and 5 Eck fistula dogs (2 determinations on each of the latter) to intravenous insulin.

SUMMARY

The sensitivity of five Eck fistula dogs to insulin (0.1 unit per kilogram intravenously) was compared to that of normal dogs. It was found that Eck fistula dogs, which have one type of chronic hepatic damage or insufficiency, are slightly but significantly more sensitive to insulin than normal dogs.

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THE INFLUENCE OF VARIOUS ANIONS OF THE LYOTROPIC SERIES UPON THE SODIUM AND CHLORIDE CONTENT OF FLUID IN THE INTESTINE

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The mechanism of absorption from the intestine to the blood is not yet thoroughly elucidated in terms of physical or chemical mechanisms. The experiments of Wells (1933) following those of Starling (1898) and Hamburger (1902) indicate without reasonable doubt that under certain well-defined conditions the driving force for the absorption of fluid from the intestine is the osmotic pressure of the proteins of the plasma. But the observations of Reid (1897) and Cohnheim (1899) that fluid passes across the gut wall from the epithelium to the serosa without hydrostatic pressure differences, with isotonic solutions on either side, cannot possibly be explained on the basis of a simple osmotic pressure difference. Goldschmidt (1921) reviewed the evidence available at the time he wrote and has listed 15 more or less distinct theories which had been advanced to explain the processes occurring in intestinal absorption. He concludes that "the mechanism of intestinal absorption is qualitatively explained by the known laws of osmosis. . . . . That factors other than osmotic may be active in modifying the process" and that "all the evidence points to their physico-chemical nature."

We have attempted to make a careful study of the relative concentrations of diffusible substances, an electrolyte and non-electrolyte, in the intestinal fluid and in the blood during the establishment of equilibrium conditions between the two. Schreinemaker (1930) has exhaustively analyzed the possibilities for setting up concentration differences across membranes with selective permeability, during the process of establishment of osmotic equilibrium. He has called attention to the fact, which was perhaps not properly appreciated before, that if the rates of diffusion of the several constituents of an osmotic system are quite different, a great many varieties of conditions may be established during the course of arrival at final equilibrium.

METHODS. The concentrations of sodium chloride and urea were determined in the blood plasma and in the fluid within the intestine at intervals of time after placing fluid containing various ions at several concentrations

in the lumen of the small intestine. In some experiments determinations were also made of the concentrations in the ultrafiltrates of the fluids under investigation. Sodium was determined by the method of Barber and Kolthoff (1928), chloride by the digestion method of Van Slyke (1923) and urea by the method of Addis (1925). Ultrafiltrations were carried on by the method described by Ingraham, Lombard and Visscher (1933). The small intestine of the dog was employed, a cannula was inserted at the proximal end of the jejunum and another at the distal end of the ileum. In some experiments the intestine was divided into four loops. The contents of the intestine were washed out by flushing with 5 liters or more of physiological salt solution at body temperature, making sure that the fluid at the end of flushing came out clear. The intestine was carefully emptied of washing fluid. The flushing out is best accomplished with the intestine as little disturbed as possible and retained within the abdominal cavity, since kinking occurs and stops the free flow of fluid when the gut is removed from the abdomen. The intestinal loops were then filled, but not distended, with the solution under investigation, as will be indicated in each experiment, and samples were withdrawn at intervals with a needle and syringe from the central portion of the loop. The animals were anesthetized with sodium amytal, 60 mgm. per kilogram body weight, injected intraperitoneally, and were kept warm by means of an infra red lamp.

RESULTS. Four types of experiments have been conducted. In the first, distilled water was placed in the lumen of the gut and the accumulation of sodium, chloride and urea was studied over time in relation to the concentrations of the same substances in the plasma. In the second type of experiment instead of distilled water approximately isotonic solutions of sodium salts of several anions were placed in the gut and the concentration of chloride determined in the blood and in the fluid in the intestine at intervals. In the third type a solution with sodium chloride content almost twice that of blood plus approximately one-half the isotonic concentration of various sodium salts was placed in the bowel, and chloride determinations made as in the other experiments. In the fourth group of experiments the properties of the various levels of the jejunum and ileum were studied by using several segments simultaneously.

1. A single example from more than twenty concordant experiments using the first method is shown in figure 1. When a steady state is approached after distilled water is placed in the intestine, the concentrations of sodium and chloride ions are each significantly higher than in the blood at the same time. In calculating concentrations in the blood the total solids have been taken into account and the figures represent the composition in milligrams per 100 grams of water. In view of the observations of Ingraham, Lombard and Visscher (1933) on the occurrence of definite concentration differences in ultrafiltrates as compared with plasma from

which they were derived, due in all probability to the membrane equilibrium effect, it seemed important to determine whether the differences in composition of the intestinal fluid and plasma could be due to the membrane equilibrium effect of the indiffusible protein anion. Ultrafiltrations of the plasma and of the fluid from the intestine (which contains some mucin after several hours) were therefore made in order to settle this question experimentally. It is invariably found, as occurred in the experiment shown in figure 1, that the differences in concentration observed between intestinal fluid and plasma are much greater than could be accounted for on the basis of a membrane effect. Moreover in the case of sodium the membrane equilibrium effect should operate to produce a lower concentration of sodium ion in the intestinal fluid than in the plasma, whereas what is actually found is a higher concentration. It must be concluded on the basis of these experiments that diffusion equilibrium with regard to

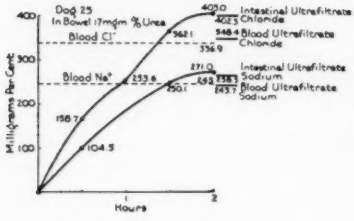

Fig. 1

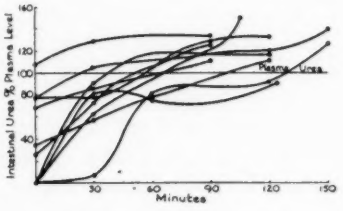

Fig. 2

Fig. 1. Sodium chloride accumulation in distilled water placed in a segment of small intestine. Concentrations in blood plasma and intestinal fluid, and in the ultrafiltrates from each, compared in milligrams per cent on the water basis.

Fig. 2. Accumulation of urea within the intestinal loop. Concentrations expressed in per cent of the blood plasma level.

sodium chloride does not exist under these circumstances between blood plasma and intestinal fluid.

Pendleton and West (1932) found a similar accumulation within the intestine in the case of urea. Our observations on the entrance of urea into distilled water in the intestine are shown in figure 2, where the composite results of the experiments are plotted. The concentration of urea rapidly rises from its initial level to, and above, the concentration in the plasma, and approaches a steady state at a figure higher than that in the water of the plasma. The ammonia content of the fluids was determined, and taken into account. The concentration differences are also apparent when ultrafiltrates are compared.

It seems likely that a type of dynamic equilibrium occurs in which the higher gut urea in the steady state is due to a faster diffusion of water than of urea from gut to blood when urea is at equal concentration in the two.

It would seem to indicate either a greater permeability of the intestinal wall to water than to urea, or a greater driving force moving the former. A similar explanation might be given for the case of the sodium chloride. Freezing point determinations of plasma and intestinal fluid show approximate identity of osmotic pressure after the first hour period. More sensitive vapor pressure measurements should, however, be made, in order to determine with certainty whether small osmotic pressure differences are responsible for the movement of the water.

2. When isotonic solutions of sodium salts of various anions are placed in the intestinal loop and the ingress of chloride ion is studied one obtains results as shown in the composite figure 3, where the concentration of chloride in intestinal fluid at various times up to 3 hours is plotted in per cent of the plasma chloride level at each time. It is seen that the citrate, the primary phosphate and the sulphate ions greatly restrain the increase in

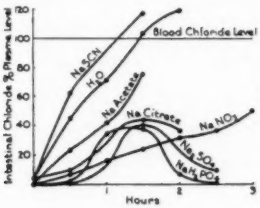

Fig. 3

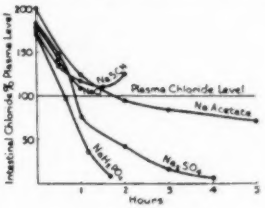

Fig. 4

Fig. 3. The influence of isotonic solutions of sodium salts of various anions upon chloride accumulation in intestinal fluid originally chloride free.

Fig. 4. The effect of half isotonic solutions of various sodium salts upon the movement of chloride from the intestinal fluid when the initial chloride level was above the blood plasma concentration.

chloride concentration. The nitrate and acetate have similar effects, but to a lower degree. The effect of sulphocyanate is different, however, and permits chloride to accumulate in the gut fluid at a somewhat faster rate than it would enter distilled water. The sulphate effect had been noted previously by Goldschmidt and Dayton (1919). It may not be particularly surprising that the rate of increase of chloride in the intestinal content is slowed by the presence of the citrate, sulphate and primary phosphate ions, inasmuch as these anions all retard the absorption of water from the gut fluid. However, the fact that in the presence of these anions the chloride concentration in the fluid within the gut actually diminishes after the first hour and a half cannot be accounted for on the basis of any simple theory.

3. In order to determine what would occur when the chloride content of the gut fluid was already high, a group of experiments was performed in which the chloride level was initially about double that in the blood. Half

isotonic quantities of sodium salts of other anions were added in the several experiments, and observations were made at intervals up to five hours. These solutions were hypertonic initially and absorbed water for the first thirty minutes, but thereafter the intestinal fluid volume decreased most rapidly in the case of NaCl and least with Na_2SO_4. Figure 4 is a composite graph of the results obtained. It is quite plain that chloride still moves or is moved from intestinal fluid when its concentration has become much less than that in the blood. It is significant that the effect follows the anion lyotropic series, being greatest for phosphate and least for sulphocyanate.

It is to be noted that the chloride ion arrives at approximately the same percentage of the blood concentration in the presence of sodium sulphate in both types of experiments in figures 3 and 4, being about 15 per cent at 3 hours. This agreement in several experiments of these types is surprising because, as will be pointed out later, the whole length of intestine is

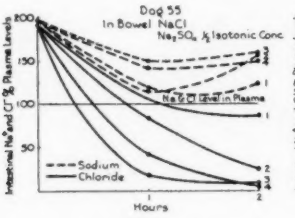

Fig. 5

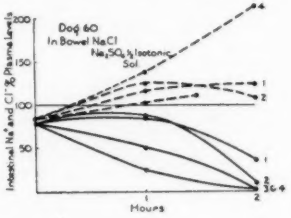

Fig. 6

Fig. 5. Sodium (broken lines) and chloride (continuous lines) concentrations in per cent of the plasma levels in four segments of the small intestine, numbers progressing aborally, with solutions of sodium chloride and sulphate.

Fig. 6. Symbols as figure 5. Original intestinal chloride slightly below the plasma level.

not equivalent in this effect, and in these experiments the whole length of small bowel was used.

The presence of NaSCN either has no effect or else has an action which is opposite to the sulphate, acetate, nitrate, primary phosphate and citrate, and favors the accumulation and concentration of the chloride in the lumen.

4. In an attempt to compare simultaneously the action of several anions on the same preparation by separating the small bowel into several loops, it was observed that the various levels of the intestine were not equivalent in their effect upon the transport of chloride and sodium ions. This phenomenon had previously been noted by Cobet (1921) in the effect of $MgSO_4$. Figure 5 shows the results of a typical experiment in which the whole small intestine was separated into four equal lengths, and each filled, after careful washing, with a solution containing approximately double the plasma concentration of Cl as NaCl, plus Na_2SO_4 in amount one-third iso-

tonic with plasma. The whole solution was initially somewhat hypertonic to the blood but became isotonic within two hours after being put into the intestine. It can be seen that the uppermost level of the jejunum causes very little chloride concentration difference, whereas the aboral portions, particularly the segments in the ileum, show profound lowering of chloride concentration. In approximate inverse ratio to the chloride dilution, is the concentration of sodium. This relationship might suggest that a membrane equilibrium governed the distribution, in which the presence of

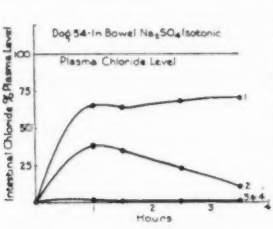

Fig. 7

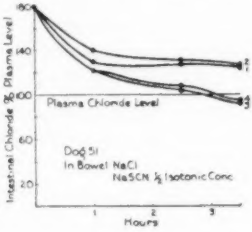

Fig. 8

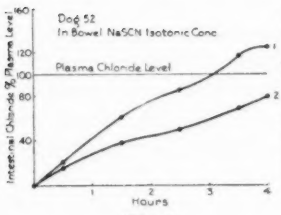

Fig. 9

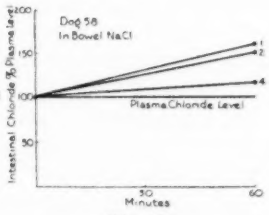

Fig. 10

Fig. 7. Behavior of the various levels of the small intestine regarding chloride accumulation in the presence of sodium sulphate.

Fig. 8. Action of the various levels of small intestine on chloride absorption in the presence of sulphocyanate.

Fig. 9. Action of different levels of small intestine on accumulation of chloride in presence of sulphocyanate.

Fig. 10. Accumulation of chloride in the several levels of intestine numbered aborally.

the $\overset{++}{SO_4}$ as an indiffusible ion brought about t[illegible] other concentration differences. In the experiment shown above the actual concentrations of $\overset{+}{Na}$ and $\overset{-}{Cl}$ in the plasma were 150 Mm. and 130 Mm. respectively. Since $[\overset{+}{Na}]_p \times [\overset{-}{Cl}]_p = [\overset{+}{Na}]_i \times [\overset{-}{Cl}]_i$, at equilibrium, if both $\overset{+}{Na}$ and $\overset{-}{Cl}$ are freely permeable in such a simple system the numerical results should indicate something as to the validity of the concept. The plasma ion product equals 19,500. The products for the several segments are as follows: (1)

21,400; (2) 7,800; (3) 2,940; (4) 1,500. Obviously the diffusion potential, as indicated by these ion products, will not account for the movement of $\overset{-}{Cl}$ from the intestinal lumen into the blood in the presence of sulphate ion.

When less than the plasma concentrations of $\overset{+}{Na}$ and $\overset{-}{Cl}$ are put into the several segments of the intestine, along with one-third isotonic Na_2SO_4, as was done in the experiment shown in figure 6, it is seen that the $[\overset{+}{Na}]$ rises and $[\overset{-}{Cl}]$ decreases, in all segments, and as noted before, most in the aboral segments and least in the proximal.

That the effect can consist in a virtual abolition of chloride influx is indicated in figure 7 where isotonic Na_2SO_4 was placed in the several segments. In the lower two segments the chloride content remained practically zero for four hours. The sodium content was not measured in this experiment.

To determine whether the different levels reacted characteristically to other anions NaSCN was employed. When the Cl content is originally considerably above the plasma level, and half the isotonic concentration of NaSCN is added, one finds, as indicated in the typical example shown in figure 8, that the lower two segments come to a lower chloride concentration than do the upper. In fact, the concentration in the aboral segments comes in $3\frac{1}{2}$ hours to a level lower than that in the plasma. When the reverse condition is studied, as shown in figure 9, where the small bowel was separated into two segments, the lower segment shows a much slower rise in chloride than the upper.

Finally, if a NaCl solution of Cl content equal to that in the plasma be put into the several segments, one sees again, as shown in figure 10, that the concentrating effect is greatest in the upper levels. The sample from the third segment was lost in this experiment, but the fourth shows a very small increase over the plasma level, while the first loop demonstrates that in one hour the Cl content of the loop fluid may be more than 50 per cent greater than that of the plasma. No determinations of freezing points were made in this last series of experiments so that it is impossible to say whether or not water was absorbed against its osmotic gradient.

Discussion. The intestine is a secreting as well as an absorbing organ, and it is therefore of importance to know how much fluid and solute may be secreted into the lumen while the fluids under investigation are being absorbed. Our attempts to quantitate this factor have failed. The intestinal juice cannot be successfully collected and measured under comparable conditions, because of its absorption. Rabinovitch (1927) attempted to account for the error due to secretion during absorption experiments by determining the alkali content of the fluid. Since this correction assumes constant alkalinity of intestinal juice, absence of alkali absorption, and

entire absence of selective reabsorption of acid anions, such as we have actually observed in these experiments, it is obvious that the total alkali content cannot serve as an unqualified measure of secretory activity. Others have employed the mucin content as a measure of secretory glandular activity. We have found the mucin to be partly precipitated by the various solutions placed in the intestine, and have concluded that homogeneous representative samples could not be obtained for such corrections.

Without a measure of the magnitude of secretion, absolute values for water and salt absorption cannot be obtained, and we have therefore chosen to confine our attention at this time to ion concentration changes during the approach to equilibrium. It is to be noted that an absolute measure of the quantitative movement of water and salt ions is necessary to an understanding of the whole problem. The observations reported in this paper do, however, demonstrate the existence of a process not properly appreciated. In fact, current views of the mechanism of absorption assume the movement of Cl from intestine to blood exclusively because of a diffusion gradient. This is obviously an incorrect view in the light of our results.

Several hypotheses suggest themselves as possible in elucidating the mechanism of the processes we have observed. It might be that water virtually free from chloride ion pours from the blood plasma into the intestine in the presence of such ions as sulphate or phosphate, and that the flow in the opposite direction, through other channels, carries whatever chloride is present into the blood. The fluid entering the intestine might be the secretion of the intestinal glands, or it might enter through other structures. On the other hand one might postulate a chemical reaction by which chloride ion would be combined in some form, perhaps by oxidation, in an organic linkage. Such a process occurring in the intestinal epithelium would serve to deplete the chloride ion concentration in the lumen. There is no objective evidence that such a process actually occurs, but if active cellular intervention occurs in absorption, it would most likely be in some such manner.

The occurrence of a typical lyotropic series in the influence of various anions is of especial interest since it indicates that some very fundamental physico-chemical mechanism is concerned in the process. It is impossible in the present state of knowledge, however, to precisely define the mechanism.

SUMMARY

1. When distilled water or hypotonic solutions of NaCl or urea are placed in the small bowel of a dog, the sodium and chloride ions and the urea accumulate and are concentrated in the bowel to a figure above their respective blood concentrations.

2. The presence of sodium sulphate, sodium dihydrogen phosphate, sodium citrate and to a less extent sodium nitrate and sodium acetate, tends to restrain the accumulation of chloride in the lumen, and causes it to leave the bowel by some means against a diffusion gradient to arrive at a concentration much below that of the blood plasma.

3. The presence of sodium sulphocyanate in the bowel affects the movement of chloride ion across the gut membrane in the opposite way from that of the above named salts, causing an accumulation and concentration in the bowel to a figure above the blood level.

4. These observations on the movement of chloride ion cannot be explained in terms of simple diffusion or osmosis.

5. The ions at the lower end of the lyotropic series have a definite effect on the movement of the chloride ion across the gut membrane, forcing it to move or be moved against the diffusion gradient. Qualitatively this may be described as indicating a unidirectional movement, but the driving force for the movement of the chloride ion is as yet unknown.

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PREGNANCY URINE GIVEN BY MOUTH TO GONADECTOMIZED RATS: ITS EFFECT ON SPONTANEOUS ACTIVITY AND ON THE REPRODUCTIVE TRACT

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It is a well established fact that in rats gonadectomy is followed by a marked decrease in general activity as well as by atrophy of the reproductive organs. It has been reported in a previous paper (Richter and Hartman, 1934) that in the spayed female these changes can be prevented by daily injections of the female sex hormone, amniotin (5 R. U. per day). And furthermore, the decrease in activity found in castrated males can be eliminated by amniotin although atrophy of the prostate gland and the seminal vesicles is not retarded. Such results would seem to indicate that amniotin possibly contains two substances, one which is effective only on the reproductive tract of females and another which modifies the activity pattern of males and females alike.

Similar experiments with theelin give essentially the same results, except that theelin seems to have a greater effect on the reproductive tract than on activity.

Since the extraction and assay of amniotin and theelin depend on the effect of these substances on the female reproductive organs, much of the activity substance may be lost in the preparatory process. For this reason it was decided to test the raw materials, pregnancy urine and amniotic fluid. The present experiments were performed with this end in view.

The materials were administered not by the usual method of injection, but by mouth in the drinking water, a very satisfactory method since the animals were found to drink either fluid in sufficiently large amounts to produce very definite effects. The following report will be limited exclusively to the results produced by pregnancy urine.

METHODS. The general procedure was much the same as that described in the paper on amniotin. The rats were kept separately in cages with revolving drums attached. The running activity, food-intake (McCollum diet), and water-intake were measured daily. Vaginal smears were studied daily and the body-weights were recorded weekly.

The animals were placed in the running cages at ages varying from 45 to 70 days but were not gonadectomized until they had reached a fairly high activity average, at an age of about 60 to 80 days. Immediately after the gonadectomy they were given pregnancy urine, and records were kept

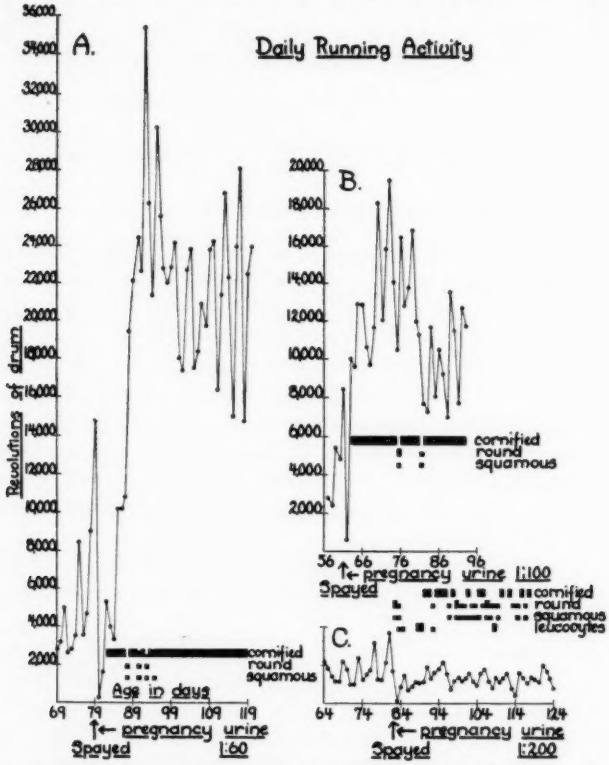

Fig. 1 A. Graph showing the effect of pregnancy urine in dilutions of 1:60 on the daily running activity of a spayed female rat; also the effect on the reproductive tract manifested in the vaginal smears.

Fig. 1 B. Graph showing the effect of pregnancy urine in a 1:100 dilution on activity and the reproductive tract.

Fig. 1 C. Graph showing the effect of a 1:200 dilution of pregnancy urine on activity and the reproductive tract.

for forty days post-operatively. Then the animals were killed and autopsied, and all the glands of internal secretion were weighed and preserved for histological study.

By means of graduated water bottles it was possible to obtain an accurate measure of the amount of urine taken per day. In order to eliminate

unpleasant odor or taste as much as possible, all of the water bottles were thoroughly cleaned and boiled every day. The urine was obtained each day from the Out-Patient Department of the Obstetrical Clinic of the Johns Hopkins Hospital. It was a composite specimen of the urine from a number of different patients in the last three months of pregnancy.

RESULTS. *Effect on activity.* That the usual marked decrease in activity which follows gonadectomy is eliminated by pregnancy urine taken by mouth is demonstrated quite definitely by the accompanying figures, in which the activity in number of revolutions of the drum is represented on the ordinates and the age of the animal in days on the abscissae.

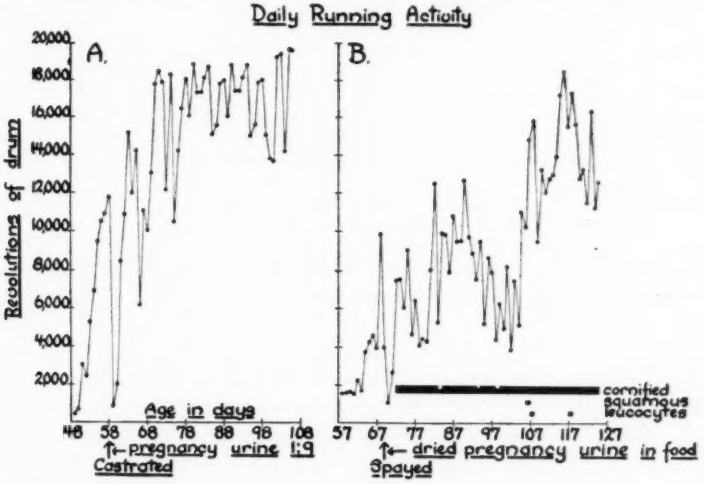

Fig. 2 A. Graph showing the effect of pregnancy urine in a dilution of 1:9 on the activity of a castrated male rat.

Fig. 2 B. Graph showing effect of dried pregnancy urine on activity and the reproductive tract. The urine was dried at 125°C. and given in the food.

Figure 1 A presents the activity record of a female rat started running at 69 days of age, gonadectomized at 79 days, and immediately given pregnancy urine in dilutions of one part urine to sixty parts of water. The animal not only failed to show the decrease in activity which characteristically follows gonadectomy, but continued to become more active so that after 15 days a very high level of activity was reached and maintained until the conclusion of the experiment 40 days after the operation. The final level of activity, about 22,000 revolutions per day, was more than ten times as high as the average for spayed rats of this age and much above the average level of 11,000 revolutions for normal females.

A similar though less marked effect is shown in the chart in figure 1 B

TABLE 1

RAT NUMBER	SEX	DILUTION OF URINE	AVERAGE DAILY RUNNING ACTIVITY IN REVOLUTIONS		HIGHEST PEAK IN ACTIVITY AFTER OPERATION	AVERAGE DAILY WATER INTAKE IN CUBIC CENTIMETERS		AVERAGE DAILY URINE INTAKE IN CUBIC CENTIMETERS	BODY WEIGHT AT END OF EXPERIMENT IN GRAMS
			Ten days before operation	Last forty days of life		Ten days before operation	Forty days after operation		
178C	♀	1:2	11,854	7,848	20,700	14.9	4.8	2.6	95
176C	♀	1:2	3,324	3,398	11,200	26.2	4.0	2.0	90
185C	♀	1:2	6,098	7,956	17,700	23.0	7.2	3.6	98
186C	♀	1:2	7,286	10,238	21,500	18.0	5.1	2.6	88
Average			*7,140*	*7,360*	*17,775*	*20.5*	*5.3*	*2.7*	*93*
210C	♀	1:4	2,404	1,555	4,600	17.5	6.0	1.5	105
205C	♀	1:4	7,666	12,133	22,300	21.1	11.0	2.7	132
204C	♀	1:4	2,407	8,602	15,600	23.8	8.8	2.2	95
198C	♂	1:4	4,605	5,499	13,300	23.5	4.7	1.2	140
Average			*4,270*	*6,947*	*13,950*	*21.4*	*7.6*	*1.9*	*118*
221C	♀	1:9	3,211	6,008	15,700	16.3	9.8	1.1	118
225C	♂	1:9	617	14,745	19,500	26.9	15.7	1.7	128
216C	♂	1:9	6,585	2,103	8,600	24.1	8.6	1.0	164
223C	♂	1:9	3,824	5,997	15,100	28.2	11.0	1.2	200
Average			*3,559*	*7,213*	*14,725*	*23.9*	*11.3*	*1.3*	*152*
252C	♂	1:15	2,060	8,579	20,100	21.1	11.1	0.7	182
232C	♂	1:15	11,484	7,598	20,100	19.7	10.5	0.7	160
234C	♀	1:15	9,059	9,936	19,600	28.6	13.7	0.9	150
248C	♀	1:15	4,125	12,097	27,200	14.7	10.1	0.7	112
Average			*6,682*	*9,552*	*21,750*	*21.0*	*11.3*	*0.8*	*151*
253C	♀	1:30	9,042	7,964	19,600	24.6	12.3	0.4	168
256C	♀	1:30	3,647	11,673	23,800	29.3	24.0	0.8	150
259C	♂	1:30	3,308	7,610	13,300	21.4	19.3	0.6	192
255C	♂	1:30	4,474	6,168	15,600	26.5	18.4	0.6	202
Average			*5,118*	*8,353*	*18,075*	*25.5*	*18.5*	*0.6*	*178*
270C	♂	1:60	4,380	4,516	9,600	20.6	21.8	0.4	186
266C	♂	1:60	4,233	7,514	15,600	32.2	23.4	0.4	202
264C	♀	1:60	7,086	12,572	19,600	27.3	18.9	0.3	140
275C	♀	1:60	5,858	19,617	35,200	38.3	24.9	0.4	167
Average			*5,389*	*11,338*	*20,000*	*29.6*	*22.6*	*0.4*	*174*

TABLE 1—*Concluded*

RAT NUMBER	SEX	DILUTION OF URINE	AVERAGE DAILY RUNNING ACTIVITY IN REVOLUTIONS		HIGHEST PEAK IN ACTIVITY AFTER OPERATION	AVERAGE DAILY WATER INTAKE IN CUBIC CENTIMETERS		AVERAGE DAILY URINE INTAKE IN CUBIC CENTIMETERS	BODY WEIGHT AT END OF EXPERIMENT IN GRAMS
			Ten days before operation	Last forty days of life		Ten days before operation	Forty days after operation		
276C	♂	1:100	1,994	2,592	5,600	23.6	20.1	0.2	240
265C	♂	1:100	1,482	667	1,500	28.7	30.9	0.3	211
261C	♀	1:100	5,849	4,826	8,700	23.1	13.8	0.2	182
283C	♀	1:100	3,906	10,919	19,400	23.0	15.9	0.2	132
Average			*3,308*	*4,751*	*8,800*	*24.6*	*20.2*	*0.2*	*191*
287C	♂	1:200	4,825	3,630	12,300	24.2	18.3	0.1	238
286C	♀	1:200	3,141	4,057	7,700	21.3	14.2	0.1	192
284C	♀	1:200	4,438	3,610	7,800	20.0	16.5	0.1	182
279C	♀	1:200	1,792	1,252	2,100	32.7	16.5	0.1	175
Average			*3,549*	*3,137*	*7,475*	*24.5*	*16.4*	*0.1*	*197*

from an animal receiving pregnancy urine in a dilution of 1:100. The absence of any effect on activity in an animal receiving pregnancy urine in a dilution of 1:200 is shown in figure 1 C.

Male rats showed essentially the same effect of pregnancy urine on activity. A typical record of the running activity of a castrated male rat receiving pregnancy urine in a 1:9 dilution is shown in figure 2 A. The average activity for the last eight days before autopsy was 16,722 revolutions per day which is well above the average for normal males.

The results of these experiments are summarized in table 1 which gives the record for each of the thirty-two animals studied. There were eight groups of animals, four (usually two males and two females) to each group receiving various dilutions of pregnancy urine, from 1:2 to 1:200.

The table gives the average daily running activity for ten days before gonadectomy and for the forty days after and also the averages for each dilution for all animals in that particular group. The highest average for the forty days of post-operative observation, 19,617 revolutions, was attained by one animal receiving 1:60 dilution. This figure is definitely above the average for normal animals of the same age. It will be noted that the daily average gradually increases as the urine is diluted, reaching a peak with the 1:15, 1:30 and 1:60 dilutions, and then with higher dilutions decreasing again to the lowest average in the 1:200 group. It is interesting to note that the average highest peaks reached after the gonadectomy were approximately the same for the 1:15, 1:30 and 1:60 groups; 21,750, 18,075, and 20,000 revolutions per day, respectively.

Of particular importance is the fact that the activity of the males showed almost as great an increase as that of the females.

Effect on reproductive tract of females. By daily studies of vaginal smears it was found that the ingestion of pregnancy urine in all dilutions from 1:2 to 1:100 brought out almost continuous cornified epithelium in the vaginal mucosa of gonadectomized females, as may be seen in figure 1, A and B. In figure 1 C it can be seen that 1:200 dilution of pregnancy urine produced cornified smears irregularly, although still more frequently than in normal animals. Furthermore, at autopsy it was found that the uteri and horns were of normal size with normally thick walls. Inasmuch as it is very difficult to measure accurately the width of the uterus, homologous parts of the uteri and horns were removed and weighed. The uterus was cut off 0.5 cm. below the point of junction of the two horns with the body of the organ, and the two horns were cut off 0.5 cm. above the same

TABLE 2

Effect of pregnancy urine on uterus

DILUTION OF URINE	NUMBER OF ANIMALS	AVERAGE WEIGHT OF UTERUS IN MILLIGRAMS
1:200	2	53
1:100	2	84
1:60	2	142
1:30	2	149
1:15	2	93
1:9	1	86
1:4	3	109
1:2	6	96
Normal	4	133
Spayed	3	26

point. The weights of this Y-shaped bit of uterine tissue for all animals included in the study are presented in table 2 according to the dilution of urine. Averages for untreated normal animals (133 mgm. with a range of 100–150 mgm.) and for untreated spayed individuals (26 mgm.) are shown at the bottom.

It will be noted that the average weights of the uteri for the dilutions 1:2, 1:4, 1:9 and 1:15, namely, 96, 109, 86 and 93 mgm., are somewhat below the normal average of 133 mgm. but still far above the spayed average of 26. The averages for the 1:30 and 1:60 dilutions, 149 and 142 mgm., on the other hand, are appreciably above the normal; and even with the high dilutions of 1:100 and 1:200 the averages of 84 and 53 mgm. respectively are still higher than the spayed level.

Effect on sex apparatus of males. In marked contrast to the effect in the females, the ingestion of pregnancy urine did not in any detectable

degree stop the atrophy of sex apparatus in the males. The seminal vesicles were very small and the prostate gland was so much atrophied and so soft that it often could not be distinguished from the surrounding tissue.

Thus it seems that pregnancy urine, like amniotin, contains at least two substances: one maintains the normal condition of the reproductive tract of the female; the other has to do with keeping the animal normally active and has an equal effect in both sexes.

Actual amount of pregnancy urine taken per day. It was found that the fluid intake of the rats is decreased by the addition of pregnancy urine to their drinking water, the degree of change being proportional to the quantity of urine consumed. The summary in table 1 of total fluid intake for each rat shows that the animals receiving 1:2 and 1:4 dilutions drank only about 5 to 8 cc. per day, 2 to 3 cc. of which represented urine, whereas those on the higher dilutions of 1:100 and 1:200 drank 16 to 20 cc., an approximately normal fluid intake containing only 0.1 to 0.2 cc. of urine.

One obvious explanation of this reduction in fluid-intake could be made on the basis of the unpleasant smell or taste of the mixture. Inasmuch as the rat is particularly sensitive to smell it would seem that the odor of the urine might be a strong factor in lowering the fluid intake curve. However, it was found that after complete removal of the olfactory bulbs in eight animals pregnancy urine decreased the water-intake as before.

A further possibility to be considered is that urine during pregnancy may contain posterior pituitary substance which would reduce the fluid intake. There is some basis for this assumption in the claims of a number of workers that posterior lobe substance occurs in the urine of eclampsia and toxemia of pregnancy (Anselmino, Hoffman and Kennedy 1932; Cushing, 1934). However, there is no evidence that posterior lobe extract is effective when given by mouth.

Effect on general health and body-weight. The health of all of the animals was good as was shown by the facts that their hair was thick and glossy, and that they were active and free from infection.

The body-weights of the animals receiving the low dilutions of urine were definitely below normal (see table 1). The average for the four females of the 1:2 group was 93 grams as compared to the normal average of 150 to 165 grams. The 1:4 group averaged 118 grams; the 1:9 group, 152 grams and the 1:15 group, 151 grams. In contrast, the 1:30 and 1:60 groups fell within normal limits, while the averages for the animals receiving high dilutions of 1:100 and 1:200 were 191 and 197 grams respectively, well above normal and near the castrate level.

The great reduction in body weight in the animals receiving the low dilutions of urine may have been caused entirely, or in part, at least, by some toxin in the urine. It seems more likely, however, that the change

was due to the sex hormone contained in the urine, inasmuch as removal of the gonads causes body weight to increase, and subsequent implantation of testicular or ovarian tissue reduces the weight to normal. Thus the marked loss of weight observed in our animals may have been due simply to the excess of sex hormone contained in the low dilutions of urine.

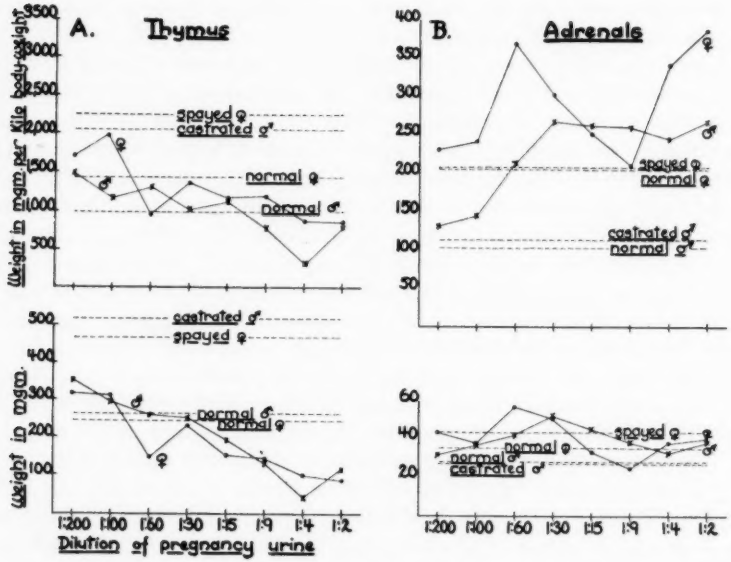

Fig. 3 A. Graph showing the effect of various dilutions of pregnancy urine on the weight of the thymus. For purposes of comparison averages are presented of the weight of the thymus of normal animals killed at about 110 days, and animals gonadectomized at 70 days, untreated, and killed at about 110 days. The lower chart gives the actual weights of the glands; the upper chart, the weights per kilogram body-weight.

Fig. 3 B. Graph showing the effect of various dilutions of pregnancy urine on the adrenal glands. The lower chart gives the actual weights of the glands, the upper chart the weights per kilogram body-weight.

Effect on the thymus. In view of the close relationship known to exist between secretions from the sex organs and the growth of the thymus, the retrogression of that organ at puberty, and its growth after gonadectomy, it was of interest to determine how the thymus was affected by the pregnancy urine. The results of these observations are summarized in figure 3 A. The lower figure shows the relation between the weight of the thymus and the dilution of the urine. The weight of the gland in milligrams is given on the ordinates, the dilution of the urine on the abscissae. For

purposes of comparison thymus weight levels are given of seven untreated animals (males and females) gonadectomized at the same age as the experimental group (70 days) and killed at the same age (110 days); also the thymus weight levels of eight normal animals neither gonadectomized nor treated, but also killed at 110 days. It can be seen that the thymuses of the untreated gonadectomized animals are almost twice as large as those of the normals. Thus, the average for the four normal males is 262 mgm. as compared to 518 mgm. for the four castrates, and the average for the four normal females is 241 mgm. as compared to 465 mgm. for the three spayed females.

It can be seen that pregnancy urine even in the dilutions of 1:200 and 1:100 produces a reduction in the weight of the thymus toward the normal, whereas in the lower dilutions it causes the thymus to decrease in weight down to one-half or one-third of its normal size. Thus, with the 1:4 dilution the average for the males was 42 mgm. as compared to 262 mgm. for normals, and for the females was 98 mgm. as compared to 241 mgm. for normals.

Inasmuch as the animals receiving the low dilutions of urine weighed much less than normal it seemed possible that the marked reduction in the weight of the thymus would reflect simply the decrease in body-weight. However, it can be seen in the graph in the upper chart, figure 3 A, which shows the weight of the thymus per kilogram body-weight at the various dilutions, that the loss of weight of the thymus is proportionately greater than the decrease in body weight.

Attention may be called further to the fact that with dilutions of 1:30, 1:60 and 1:100 the thymuses are approximately normal in size; with the higher dilution the thymus is larger, showing the effects of gonadectomy, while with the greater amount of urine the thymus is smaller, showing the effect either of an excess amount of hormone or of a toxin. It is of interest to note that these same dilutions produced the most normal conditions in activity, body-weight and size of uterus.

Effect on the adrenals. Pregnancy urine had an opposite effect on the adrenals which became larger rather than smaller, as may be seen in the graph in figure 3 B. Thus, in the lower figure which shows the level for the normal and gonadectomized males and females, the weights of the adrenals of the treated animals were approximately normal, even with the low dilutions of urine. In other words, they continued to gain despite the great loss in body weight. This may be seen more clearly in the top figure showing the weights of the adrenals per kilogram body-weight. Again for purposes of comparison, the weights are given of the adrenals of the four normal males and four normal females, and the four castrated males and three spayed females. It can be seen that the increase is directly proportional to the dilution of urine, being greatest with the 1:2 dilution,

when the adrenals for the females were 388 mgm. per kilogram body-weight as compared to 206 mgm. for normals, and for the males were 269 mgm. per kilogram body-weight as compared to 105 mgm. for normals. In other words, they were about twice as large as the normals. Although it would appear that the effect was greater in the females than in the males, the differences were very small.

Thus, the effect produced by pregnancy urine on the adrenals is opposite to that produced on the thymus, the adrenals becoming larger as the thymus regresses. Examples of such an inverse relationship between thymus and adrenals is not infrequent in literature, and others have reported that the thymus increases in size after the adrenals are removed (Marine, Manley and Baumann, 1924.) It is an accepted post-mortem finding in myasthenia gravis that the thymus is larger while the adrenals are smaller than normal.

Histological study of the gland showed that the increase in size was due to a hypertrophy of the medulla, the cells of which were enlarged and contained larger nuclei. This is in contrast to the hypertrophy of the cortex found in conditions of inanition or chronic infection and intoxication.

Effect on the pituitary. It can be seen in figure 4 A that pregnancy urine had much the same effect on the pituitary as it did on the adrenals. The graph in the lower figure shows that the weight of the pituitary was the same with all dilutions, that is, approximately normal, despite the large decrease in body weight. This increase per kilogram body weight with the lower dilutions is shown clearly in the graph in the upper figure.

Effect on the thyroid. Pregnancy urine had much the same effect on the thyroid as on the adrenals and the pituitary, as may be seen in the graph in figure 4 B. With the 1:2 dilution the thyroid weighed almost twice as much per kilogram body weight as it did in the normals. Here, the most effective dosage would appear to lie between 1:30 and 1:15 inasmuch as with the former dilution the thyroid is still slightly below normal, and with the latter slightly above.

The observations that the adrenals, pituitary and thyroid were all normal in size and that also the activity of the animals was approximately normal, are in agreement with our previous findings that these three glands are all essential for the maintenance of normal conditions of the activity mechanism (Wang, 1923; Richter and Wislocki, 1930; Richter, 1933).

Discussion. In these experiments it was found that castrated and spayed rats could be kept normally active by administration of untreated pregnancy urine by mouth, even in very minute doses of 0.1 cc. per day or less.

The question arises, then, as to which one or ones of the many different substances known to be contained in pregnancy urine would bring about this result. All of the evidence at hand indicates that the mainte-

nance of a normal amount of activity is due to a substance very closely associated with a secretion from the ovaries or placenta rather than from the pituitary. In the first place, it is a well-known fact that oestrin is effective by mouth, while anterior pituitary lobe substances are not; further, that the activity is still present after the anterior lobe substances have been destroyed by heating to 125°C. and drying as may be seen in figure 2 B; finally that the administration of the urine (even 3–4 months' pregnancy urine) to inactive animals has a retarding rather than

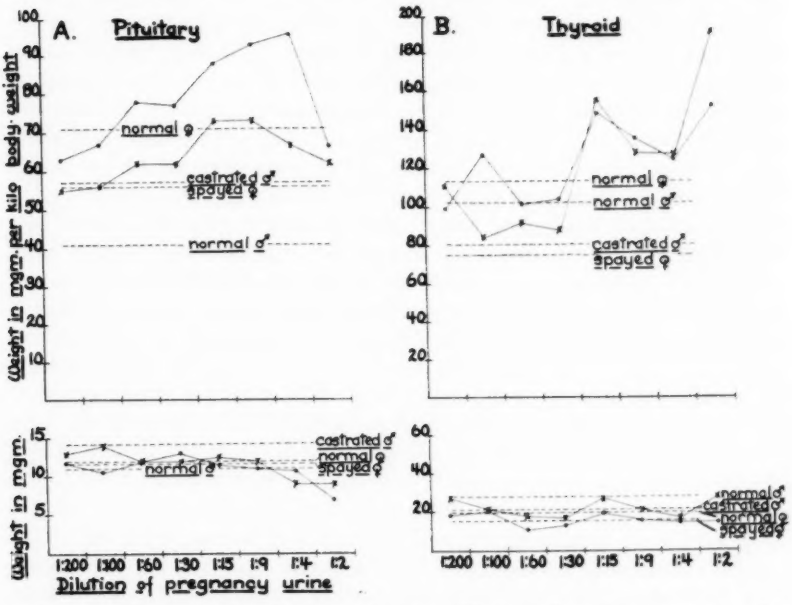

Fig. 4 A. Graph showing the effect of various dilutions of pregnancy urine on the weights of the pituitary gland.

Fig. 4 B. Graph showing the effect of various dilutions of pregnancy urine on the weights of the thyroid glands.

an accelerating effect on the growth of the sex apparatus in the males whereas it produces a very rapid maturation in the females (2–4 days). We hope to throw further light on any possible rôle played by the pituitary by a study of the effects produced by administration of urine from women with both ovaries removed.

It occurred to us that the maintenance of the high level of activity in the castrated animals might be due to an increased metabolism produced by a secretion of the thyroid gland contained in the urine. This possibility was tested out by administration of thyroid extract in the food of gonadec-

tomized rats in quantities known to produce a definite increase and return to normal level in thyroidectomized animals (Richter, 1933). These experiments were entirely negative. So also were experiments in which stimulants, cocaine and strychnin, were used.

The decrease in water-intake or partial water-deprivation may in some way have contributed to the increase in activity, and also to the changes in the glands of internal secretion, particularly to the reduction in size of the thymus. Preliminary experiments on the effect of water deprivation alone, however, have given very inconstant results although there is some indication that a part of the increase in activity and decrease in size of the thymus may be attributed to this factor. The fact that gonadectomized animals become normally active again when the pregnancy urine is administered in dried form in the food, involving little or no reduction in water-intake, indicates very definitely that the water-deprivation plays only a minor rôle. The results of a more extensive investigation of this phase of the problem now being carried on will be reported later.

The evidence, then, seems fairly definite that the maintenance of the normal amount of activity is due largely to a substance very closely associated with oestrin. Inasmuch as the effect was produced when the substances were taken by mouth, it would seem likely that it was due to theelol rather than the theelin, the latter being less effective when given by mouth.

The observation that the urine was effective when given in such minute quantities still remains very astonishing, even when full consideration is given to the fact that by giving the urine in the drinking water the substance was administered in small quantities throughout the day (7–11 times) rather than in a single dose; also when consideration is given to the observation that amniotin given by stomach tube to spayed monkeys was only about one-fifth as effective in restoring the normal condition of the reproductive tract as when given by injection (Morrell, Powers, Varley and De Frates, 1930). According to Zondek, (1929) Schoeller, Dohrn and Holweg, (1930), Morrell, Powers, Varley and De Frates, (1930), and others, oestrin given by injection is 4 to 7 times more potent than when given by mouth.

The present observation that whole untreated urine can be given without any untoward effects is in keeping with the observations made by Zamkoff (1929) and after him, Mazer and Goldstein, (1932) who have held that very good results can be obtained by injection of whole untreated pregnancy urine into women with various reproductive tract disturbances.

It is hoped that it will be possible by this technique to isolate the substance which affects primarily the activity mechanism and that this substance might be of use in the treatment of conditions of inactivity seen so frequently in psychiatric clinics.

Remarks. Preliminary comparisons made of the effects produced by

urine from normal and pregnant women on the vaginal smears suggest that there may be a basis here for a significant test for pregnancy.

It was found that urine of over one hundred women, 7 to 9 months pregnant, administered to spayed adult female rats brought out cornified cells in the vaginal smears in 2 to 3 days. These positive results were observed for dilutions as high as 1:100 and 1:200. Even more striking were the results obtained when the urine was administered to immature female rats. Normally the vagina does not open until the 35th day or later; however, with the pregnancy urine it opened as early as the 17th day, even when the urine was administered for only two days.

Also urine from normal women was tested in all strengths, varying from a concentration of $\frac{1}{10}$ of its original volume to dilutions of 1:15. It was found that urine from normal women concentrated to $\frac{1}{5}$ its volume occasionally brought out cornified smears in a few of the animals, but when given unconcentrated it consistently failed to do so.

In other words, when the actual amount of the urine intake was considered it was found that it required approximately 1000 times as much urine from normal women as from pregnant women to bring out cornified smears and to open the vagina.

Positive results were obtained from urine of women $1\frac{1}{2}$ months pregnant. So far there has not been an opportunity to test out many women in these early stages.

It may be pointed out that this method has the obvious advantage over the usual Ascheim-Zondek test in that besides giving 100 per cent results, it requires no surgery and no special chemical technique.

SUMMARY

1. The urine of women 7 to 9 months pregnant, given daily in drinking water, keeps up a normal level of activity in castrated male rats and in spayed females.

2. It produces this result when given in dilutions varying from 1:2 to 1:100. In the very high dilutions the rats received less than 0.1 cc. of pregnancy urine per day.

3. Pregnancy urine even in the high dilutions maintains the reproductive tract in a normal condition in spayed females, as shown by the vaginal smears and autopsy findings.

4. It does not in any way stop the atrophy of the sex apparatus of castrated males.

5. It is concluded that pregnancy urine contains two substances, one which maintains the normal condition of the reproductive tract in the female and has no effect on the sex-apparatus of the male, and another which maintains a normal level of activity in male and female alike.

6. Pregnancy urine causes a very rapid retrogression of the thymus while the adrenals, pituitary, and thyroids either reach the normal size or actually become hypertrophied. This is in agreement with the previous demonstration of the importance of these three glands for the maintenance of the normal amount of activity.

7. There is some indication that the method may be of use as a test for pregnancy.

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